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# BOTANICAL GAZETTE

*OCTOBER 1907*

## THE CHRYSANTHEMUM RAY BLIGHT[1]

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(WITH FIFTEEN FIGURES)

The common name chosen for the disease at present under discussion is taken from the most conspicuous symptoms of the malady, a blighting of the corolla of the ray flower, resulting in poorly developed, discolored, one-sided heads (*fig. 1*).

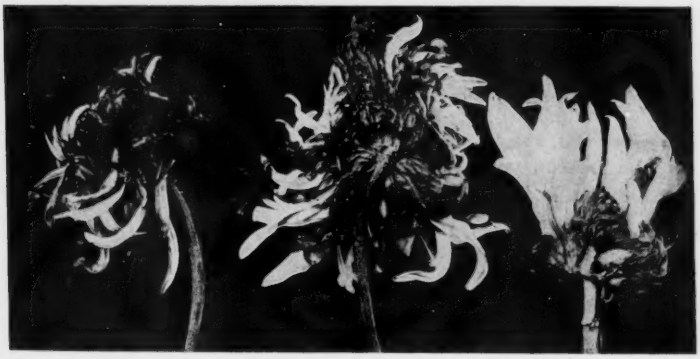


FIG. 1.—Three heads, showing disease as it naturally occurs.

Attention was first called to the disease by a letter from Fayetteville, N.C., dated November 13, 1906, and accompanied by specimens. The disease has been known at that place and has recurred for three years, each year with increasing destructiveness. It was first attrib-

[1] Contribution from the Laboratory of the North Carolina Agricultural Experiment Station, Raleigh, N. C.

uted by the owner of the plants to want of ventilation, but upon its recurrence the second year, the possibility of such a cause was precluded by the conditions. The disease was found upon plants in the open as well as upon those of the greenhouse. The first year it was found only upon late varieties, but this year it appeared upon all varieties in the owner's collection, the variety "Nellie Pockett" being particularly susceptible. The "Golden Wedding," a late yellow variety, always suffers badly in the collection in question. Since receiving the specimens from Fayetteville the disease has been found in Raleigh, and it may be of wide distribution.

## SYMPTOMS

The disease is most conspicuous in the flower clusters, which it attacks usually upon one side, either in the bud or during various stages of blooming. The affected blossoms turn straw color or brownish, cease to develop, and wither, the discoloration proceeding from base toward tip on each individual flower, thus distinguishing the disease at once from many disorders which may resemble it superficially. If the case be severe, and a bud be attacked while still young, no rays will develop; the head will not open. If the attack be later, a portion of the head, one-half or two-thirds, or more or less, may develop normally and thus by contrast heighten the conspicuousness of the disease. Heads nearly open and apparently healthy may on the following day show one side far advanced in disease. The owner says, "it developed so fast that a large perfect bloom in the morning would be wilted by night."

The receptacle turns black, and the peduncle may likewise be blackened to a distance of one or two centimeters below the head. Later, shriveling and softening of the peduncle allow the head to nod in a characteristic manner.

On the affected plants the stems are often blackened for several centimeters, in a band more or less completely encircling the stem. Frequently this diseased region is associated with a leaf, the petiole of which also partakes of the discoloration.

## MICROSCOPIC EXAMINATION

Under the microscope, diseased flowers, rays, corollas, receptacles, peduncles, and the diseased portions of the stem all reveal

the presence of a rather coarse, much branched mycelium that is easily recognized by its septa, which are numerous and stand out with an especially striking clearness (*fig. 2*). In the humid region between the flowers in the head, the mycelium becomes aerial, forming a loose floccose weft, visible to the naked eye. In the pith cavity of the blighted receptacle a similar profuse mycelial development occurs; while on old specimens, in culture dishes, pycnidia were also found identical with those which developed in the pure cultures to be described later (*fig. 3*). Only one fungus was seen and no bacteria whatever were visible.

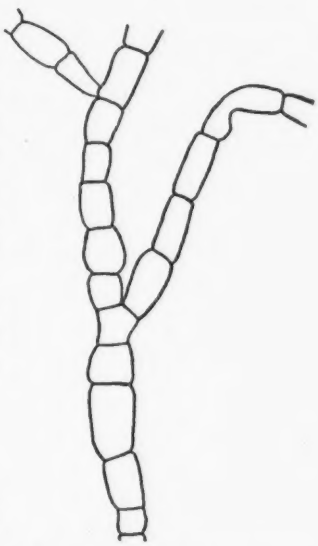


FIG. 2.—Normal hyphae, mature, showing septation with slight constriction; also the mode of branching.

### ISOLATION

Diseased ray flowers were removed with sterile forceps and thrown upon solidified pea agar in Petri dishes, several to each dish. Similarly, with aseptic precautions, bits of the wood and bark from diseased peduncles, stems, and from the inside of the receptacle were plated. Each of these cultures, 25 or more in number, plated on November 18, resulted within 24 hours in pure colonies, all alike, of a fungus with a mycelium identical in appearance with that seen in diseased tissue.

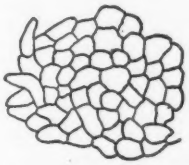


FIG. 3.—Surface view of pycnidium showing reticulation.

### THE FUNGUS

The colonies grew with remarkable rapidity and remained sterile until five days old. Pycnidia were found then in the oldest portions of a colony upon one of the plates. They were amber color, as seen with the two-thirds objective and transmitted light approaching no. 30 of SAC-

CARDO's *Chromotaxia*, or the YYO[4] of the PRANG *Standard color scale*. They were coarsely reticulated on the surface (*fig. 3*), owing to their composition of woven, coarse, mycelium threads. In diameter they ranged from 102 to 204 $\mu$ (the most usual size being about 150 $\mu$). They were round in outline and elevated from the surface, hemispherical. Grown upon corn and also upon the apple agar, the pycnidia were very much darker, almost black. The ostiolum seemed entirely absent in many pycnidia which developed deep in the culture medium, but was present in all growing upon the surface of artificial media or upon natural substrata.

FIG. 4. — Spores from pycnidia grown on cow pea agar.

The spores are regular and oblong, or quite irregular-oblong, or swollen and rounded at one end and pointed at the other. Regular-

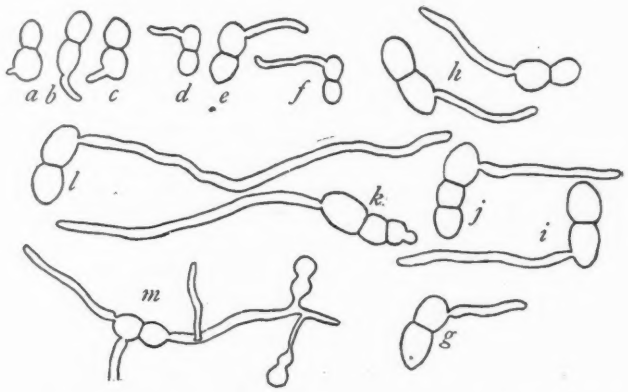
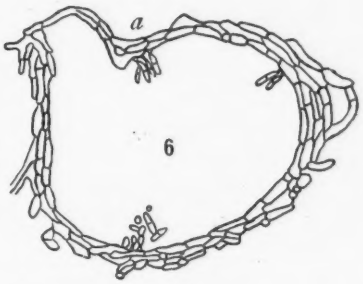



FIG. 5.—Stages in the germination of spores; *e* and *f*=*c* and *d* respectively, drawn one hour later; *c* and *d* drawn at 6 hours of age, and *e* and *f* at 7 hours; *m* 15 hours old.

oblong, however, is the prevailing type, with a pronounced tendency to irregularity (*fig. 4*). One septum, more easily seen in stained preparations, is visible in most of the older spores; though young or small spores and many larger older ones do not show it. The septum is frequently central, but there is a tendency for it to be located

nearer to one end than to the other. Rarely two septa or even three are present in one spore. The spores measure from 3 to 6.2 $\mu$ in thickness by 10 to 20 $\mu$ in length; 6.2 $\mu$ by 18 $\mu$ being the usual size. The spores are hyaline or very slightly greenish. In mass, as extruded from ripe pycnidia, they appear slightly pink. The walls are evidently mucilaginous, since the spores, as they are pushed from the pycnidia upon the absorption of water, issue in worm-like coils, cohering thus for considerable time, though they are not in visible contact. The spores germinated rapidly in water (*fig.* 5), practically every spore germinating. In germination the septum appeared in all spores, and one to three germ tubes protruded from each cell. These tubes rapidly became richly septate, and the protoplasm highly vacuolate.



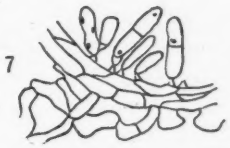




FIG. 6. Section of pycnidium. *a*, neck of ostiolum.—FIG. 7. Portion of pycnidial wall in section showing method of spore attachment.

In sections (*fig.* 6) the spores are seen to be borne on all inner portions of the pycnidial wall except the very short neck of the ostiolum, each spore originating singly (*fig.* 7) on the end of a very short stalk (about 2 to 5 $\mu$ long). These sporophores are lateral branches of the mycelial threads which constitute the pycnidial wall. The pycnidium originates within the host tissue, i. e., immersed, but rapidly enlarges to burst the overlying epidermis, so that the mature or even half-grown pycnidium appears to be superficial. There is no special aggregation of mycelium to be found below the base of the pycnidium.

The very young pycnidium is solid. Later a spore cavity develops, though apparently no more rapidly than is necessitated by spore production. In the youngest pycnidia having a spore cavity, the sur-

rounding wall is composed of several (five to eight) layers of mycelium. As the spore cavity becomes distended the wall becomes thinner, until at maturity it is composed of only one, two, or three layers of mycelium, while the region of the ostiolum becomes one layer thick and eventually ruptures.

This fungus clearly belongs to the section HYALODIDYMAE Sacc. of the SPHAERIOIDEAE Sacc. and seems closest in affinity to the members of the genus *Ascochyta*. The possession of a slight beak, which however is always very small and often entirely absent, might throw it into the genus *Rhyncophoma* Karst., but members of that genus are typically upon wood, usually saprophytic, and have a more pronounced beak.

Since no Ascochyta, or indeed any other species of the Sphaerioideae Hyalodidymae, which could by any possibility be identical with the species considered in this paper, has come to my notice as growing upon the chrysanthemum or any of its kin, I regard this as a new species, for which I give the following description.

**Ascochyta Chrysanthemi,** n. sp.—Perithecia few, immersed, early erumpent, single or scattered, round, hemispherical, amber colored, 100–200 $\mu$, mostly about 150 $\mu$; ostiolum central, small, dark bordered, often raised by a short neck; surface reticulate, pycnidia on agar media irregular, often with two ostioles and varying much in size, black in color. Mycelium abundant, innate, also superficial, aerial, floccose, richly septate.

Spores oblong, straight or irregular, 3 to 6.2×10 to 20 $\mu$, mostly 6.2×10 $\mu$, ends obtuse or acute; septum usually one, often obscure, rarely 2 or 3, usually without constriction until germination; protoplasm vacuolate, hyaline or light pink in mass.

HABITAT: in corollas, heads, petioles, and stems of cultivated plants of *Chrysanthemum indicum*, causing blight. North Carolina.

## CULTURE CHARACTERISTICS

*Thermal relation.*—Cultures in Petri dishes on cow pea agar were placed in darkness at three temperatures with the results appearing in the adjoining table.

The growth both outdoors n the cold, and in the incubator, was exceedingly irregular, stunted, or slow, while that of room temperature was very much more rapid.

In addition to being an unfavorable temperature for growth, the heat of the incubator produced great irregularity in the zonal development; threads started vigorously, stopped suddenly, and growth began in another direction, so that all regularity of zone formation was lost. Much of the mycelium also became incased in a dark coat, as described under the effect of cow pea leaf agar below. The plates kept at outdoor temperature, while they maintained a fair regularity in the formation of zones, did not make the same number of zones as the cultures kept at room temperature, the number of zones made in the room temperature being six while that outdoors was four.

*Acid relation.*—Cultures in lettuce agar in the dark at room temperature in various degrees of acidity gave the record shown on Table II on the following page:

TABLE I

INOCULATED DEC. 8, 1906. GROWTH IN MM.

| December | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | Daily average |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| In room | | | | | | | | | | | | |
| Temp. max./min. | —/— | —/— | 12/20 | 12/20 | 18/21 | 17/21 | 18/20 | —/— | 12/18 | —/— | 11/17 | 16.19 |
| Plate 1..... | 2 | 4 | — | 12 | 15 | 18 | 22 | 25 | 26 | 33 | 39 | 3.5 |
| Plate 2..... | 2 | 3 | — | 12 | 15 | 18 | 22 | 25 | 26 | 33 | 39 | |
| In incubator | | | | | | | | | | | | |
| Temp. 30–35° | | | | | | | | | | | | |
| Plate 35..... | 3 | 3 | | | contaminated | | 8 | 11 | 15 | 22 | 25 | 2.2 |
| Plate 36..... | 3 | 3 | | ? | ? | | | | | | | |
| Outdoors | | | | | | | | | | | | |
| Temp. max./min. | 3/17 | 10/18 | 1/14 | 3/7 | 1/16 | 6/22 | 9/22 | 10/21 | 22 | 14/14 | —/— | —/— |
| Plate 37..... | 0 | 2 | | ? | | | 7 | 11 | 14 | 19 | 22 | 2.0 |
| Plate 38..... | 0 | 2 | | | | | 7 | 11 | 14 | 19 | 22 | |

TABLE II

INOCULATED DECEMBER 13. GROWTH IN MM.

| Plate | Drops of normal acid or alkali per tube | Fuller's scale | 14 | 15 | 16 | 17 | 18 | 19 | Average per day |
|---|---|---|---|---|---|---|---|---|---|
| 25.... | acid 10 | +40 | ? | 0 | 0 | | | | |
| 27.... | acid 5 | +20 | ? | 0 | 0 | | | | |
| 49.... | 0 | 0 | 2 growth | 3 | 7 | 12 | 15 | 18 | 3.1 |
| 31.... | alkali 5 | −19 | | 3 | 5 | 11 | 14 | 16 | 2.6 |
| 29.... | alkali 10 | −39 | ? | 2 | 3 | 8 | 9 | 10 | 1.6 |
| 34.... | alkali 15 | −59 | ? | 0 | 0 | 2 | 3 | 4 | .7 |
| 33.... | alkali 20 | −99 | ? | 0 | 0 | gr. | 5 | 6 | 1. |

It is clearly seen from these data that a neutral medium or one only slightly alkaline favors the growth of this fungus.

In the plates with 10, 15, and 20 drops of normal alkali there was no formation of zones at all. In the plate bearing 5 drops of alkali two zones were formed.

The four cultures inoculated December 8, grown at room temperature, two in light and two in darkness, agreed almost absolutely in rate of growth, a total difference of 1 or 2$^{mm}$ noted at the end of ten days' growth being so small as to be insignificant.

*Growth in various media.*—Cultures in agar with various nutrients at room temperature in the dark grew as follows:

TABLE III

INOCULATED DECEMBER 13, 1906. GROWTH IN MM.

| Plate | Medium | Dec. 14 | Dec. 15 | Dec. 16 | Dec. 17 | Dec. 18 | Dec. 20 | Dec. 21 | Jan. 12 |
|---|---|---|---|---|---|---|---|---|---|
| | | 17–21° | 18–20° | | 12–18° | | | | |
| 9... | Pure agar | Grew | 4 | 6 | 11 | | | | 65 |
| 10.. | | " | 4 | 7 | 11 | 14 | | | 70 |
| 7... | Pea agar | " | 4 | 6 | 10 | 13 | | | 18 |
| 8... | | " | 4 | 6 | 10 | 14 | | | 18 |
| 15.. | 1% peptone | " | 3 | 5 | 9 | 9 | | | 70 |
| 16.. | agar | " | 4 | 7 | 10 | 11 | | | 70 |
| 11.. | 1% glucose | " | 5 | 9 | 15 | 19 | | | 80 |
| 12.. | agar | " | 5 | 8 | 13 | 17 | | | 80 |
| 13.. | 5% glucose | " | 6 | 11 | 16 | 21 | | | 70 |
| 14.. | agar | " | 6 | 11 | 16 | 21 | | | 75 |
| 17.. | 1% starch | " | 4 | 6 | 10 | 11 | | | |
| 18.. | agar | " | 4 | 7 | 11 | 12 | | | |

| Plate | Medium | Dec. 8 | Dec. 9 | Dec. 10 | Dec. 11 | Dec. 12 | Dec. 13 | Dec. 14 | Dec. 15 | Jan. 12 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 12–20° | 12–20° | 18–21° | 17–21° | 18–20° | |
| 5... | Standard | Grew | 1.5 | 2 | 5 | 9 | 10 | 11 | 14 | 52 |
| 6... | beef agar | " | 1.5 | 2 | 5 | 10 | 12 | 13 | 15 | 32 |
| 3... | Cow pea | " | 2 | 3 | 5 | 12 | 14 | 18 | 22 | |
| 4... | agar | " | 2 | 3 | 5 | 12 | 14 | 18 | 22 | |
| 39.. | Standard | " | 2 | 2 | 4 | 6 | 8 | 11 | 15 | 35 |
| 40.. | beef gelatin | " | 2 | 2 | 4 | 6 | 10 | 12 | 15 | .. |

Colonies grown in *pure agar* in dark or light and those upon pea agar were of the same general appearance, thin, colorless, and structureless, zone formation being entirely absent. A very few pycnidia were produced. By increasing the richness of the pea agar to 4 per cent., pycnidia were secured, but without change in other characters. The growth in pea agar was no more rapid than that on pure agar, but was much more dense; the mycelium was colorless and very few pycnidia developed.

One per cent. *peptone* adds nothing to the nutrient value of the medium for this fungus. Growth comes to an end earlier than in pure agar or other media; there are no pycnidia, no color to the mycelium, and only a very slight indication of zonation.

*Glucose*, 1 and 5 per cent., favor growth, the five per cent. being slightly better than the one per cent. No pycnidia developed, but there is marked, though irregular, zonation in both darkness and light, and the whole mycelium develops a striking, intense blackness. The septation and cell contents are normal and the color rests with the cell wall.

*Starch*, 1 per cent., did not increase the rate of growth, although upon microscopic examination it was clear that the starch grains were digested almost as soon as the advancing threads of the fungus reached them, and growth continued long in these plates. The effect of starch upon the general appearance of the colony and upon the mycelium was precisely as in the glucose plates.

In *standard beef agar* and *gelatin*, growth was about as with peptone agar, though it continued for a longer time. The mycelium was of abnormal, irregular character (*fig. 8*), and there were no pycnidia nor any development of color nor of well-marked zones. On *beef*

*gelatin* the growth was more uniform than on beef agar, and the zones were somewhat clearly marked, liquefaction following in the wake of the mycelium, lagging about 4 or 5$^{mm}$ behind the mycelial tips.

In *litmus lactose agar* the colonies developed as on beef agar without acidity.

Upon *cow pea leaf agar* the growth, while exactly parallel in rapidity with the growth upon the cow pea agar, exhibited remarkable differences in the structure of the colony, the number of zones being much less and the breadth consequently much greater. The mycelium also clung

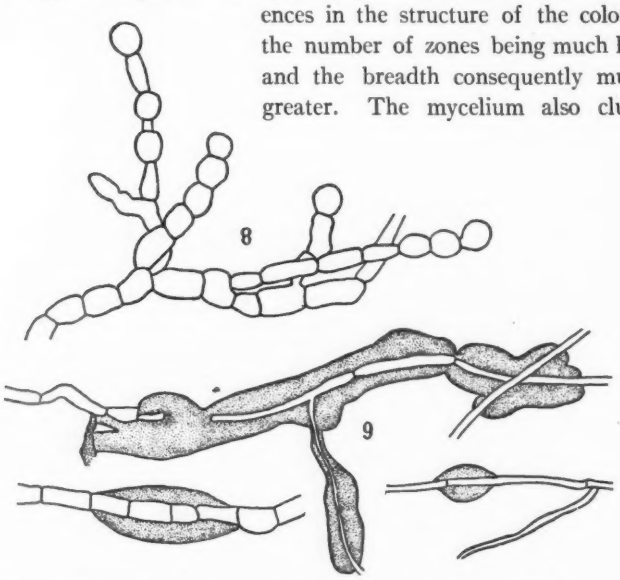



FIG. 8. Abnormal hyphae, mature, as grown in gelatin, the cells being unusually swollen.—FIG. 9. Thickening of hyphae, produced by too high temperature or an excess of nitrogenous food.

closer to the medium, was less floccose and less aerial, and in the older region of the mycelium the cells became extraordinarily thick and short and assumed a dark color. In some instances the protoplasm within the cell rounded off and became invested by a thick brown coat. In other instances a similar brown coat of much greater thickness, often four or five times as thick as the diameter of the mycelium, would form around the mycelial threads (*fig. 9*). Cow pea agar proved the best medium for the growth of this fungus. The

concentric zones formed with great regularity, five dense zones and six lesser zones being formed in a period of eleven days. In the older central portion pycnidia began to form at the end of about eight days, though often later. Pycnidial formation proceeds from the central zones to the younger zones, the pycnidia being most numerous in the denser zones, although they are by no means lacking in the lesser zones.

Upon *boiled rice*, in test tubes, the fungus grew luxuriantly. As the threads first invaded the rice there was developed a salmon color, which later turned to black.

Upon *boiled corn*, in test tubes, the mycelium developed well, became black, and pycnidia were present in abundance at the end of eleven days.

The most striking features noted in these nutrition studies were the influence of starch and glucose in agar or the starch of the rice or corn media in causing the development of an intense black coloration in the mycelium; and the unfavorable influence of peptone, beef, and gelatin as evidenced by distorted mycelium and the failure to develop pycnidia.

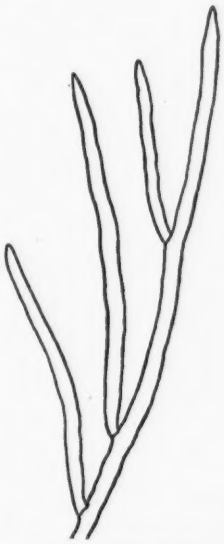


FIG. 10.—Tips of young rapidly growing hyphae.

The *formation of zones* is the most characteristic feature of growth of this fungus upon clear, solid media (*fig. 11*). Reference has constantly been made to this feature. The lesser zone, the zone which immediately surrounds the point of inoculation, is due to the mycelium growing out rapidly in every direction from the point of inoculation. As each individual thread becomes more and more distant from the center and has made more and more branches, the threads begin to crowd each other much more closely until, if conditions be proper, a time is reached when apparently the crowding becomes so great that further growth in length is inhibited. There then occurs a development of aerial hyphae and a thickening and darkening of the

ends of hyphae already produced; this results in a darker zone surrounding the lighter zone. After maintaining this condition for some time, individual threads, in a scattered fashion, break through the outer dense zone, continue their growth, and proceed to the formation of a second lesser zone, followed in course of time by a second denser zone, all being formed in precisely the same fashion that the first two zones were made. Plates have been exposed under different light relations and different temperatures, and it seems probable that

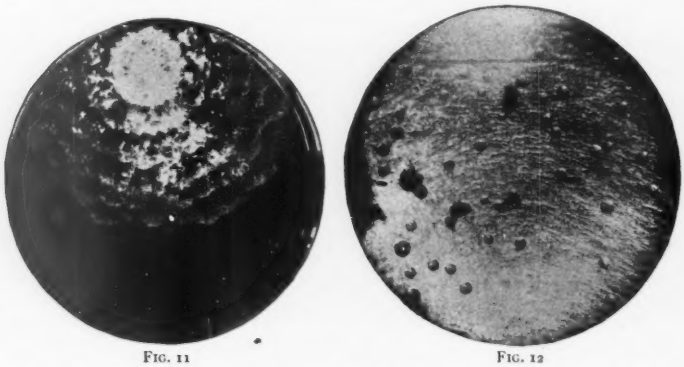


FIG. 11 FIG. 12

FIG. 11. Pure culture of the fungus 17 days old, grown upon cow pea agar in diffuse light; pycnidia not then formed.—FIG. 12. Pycnidia on agar plate, viewed from below.

there is no connection between either of these factors and formation of zones in this fungus, other than is occasioned by increasing or decreasing growth.

## INOCULATIONS (*figs. 13, 14*)

Some forty inoculations were made with mycelium taken from pure cultures on bits of agar. Of these twenty-eight gave positive results. Control cultures remained uninfected. Inoculations were made within the involucral scales, between the flowers in the head, on stems, and on leaves, as follows:

### *Series I*

Inoculated from mycelium obtained from the original agar plate culture on which diseased rays had been placed. Inoculations made November 22, 1906.

*Inoculation no. 1:* under the involucre of the bud which was just beginning to open; December 8, growth observed; December 18, exhibited typical disease.

*Inoculation no. 2:* in the axil of the uppermost bract, in needle pricks, with agar; the bud above was just beginning to open; examined December 8 and 18 with no indication of disease.

*Inoculation no. 3:* with mycelium upon agar upon the end of the unopened bud; examined December 8, no growth; December 18, typical disease.

FIG. 13.—Four diseased heads produced by inoculation from pure culture.

*Inoculation no. 4:* control cultures similar to no. 3 with sterile needle; examined December 8 and 18, no growth.

*Inoculation no. 5:* at base of leaf; the tissue unbroken; examined December 8 and 18, no result.

*Inoculations nos. 6, 8:* agar laid upon the outside of the involucre; tissue uninjured; examined December 8 and 18, no results.

*Inoculation no. 7:* lost through accident.

*Inoculation no. 9:* at the base of the petiole; tissue uninjured; examined December 8 and 18, no result.

*Inoculation no. 10:* leaf base pricked and inoculated with agar culture; examined December 8 and 18, no results.

It is to be noted that in this series nos. 1 and 3 produced typical disease; that is, all of the inoculations which were attempted within

the involucre. Control inoculations produced no growth, and attempts to produce the disease upon leaves and stems through uninjured tissue failed.

*Series II*

Inoculated November 24, 1906, with mycelium which was richly set with pycnidia.

*Inoculations nos. 11, 12:* agar bearing mycelium placed deep between the petals of the open flower; examined December 8, showed typical disease.

*Inoculation no. 13:* same as no. 11 using sterile agar; control inoculation; examined December 8 and 18, no growth.

*Inoculation no. 14:* peduncle immediately below the bud scarified and inoculated with mycelium on agar; examined December 8, typical disease.

*Inoculation no. 15:* similar to no. 14, but with sterile agar; control experiment; examined December 8 and 18, no growth.

*Inoculation no. 16:* upon scarified stem immediately under head with agar bearing mycelium, the whole covered with wet cotton; examined December 8 and 18, no result.

*Inoculation no. 17:* conditions same as in no. 16; examined December 18, head typically diseased.

*Inoculation no. 18:* conditions same as in no. 16; examined December 8, no result; December 10, typical disease.

*Inoculation no. 19:* agar bearing mycelium placed upon the tip of the opening head; examined December 8, no growth; December 18, typical disease.

*Inoculation no. 20:* conditions same as in no. 19; examined December 8, typical disease.

*Inoculation no. 21:* conditions same as in no. 19; lost through accident.

It is to be observed that in this series the inoculations within the flower produced typical disease. Control inoculations produced no disease. Inoculation upon scarified stem when covered with damp cotton to prevent evaporation was capable of inducing the disease.

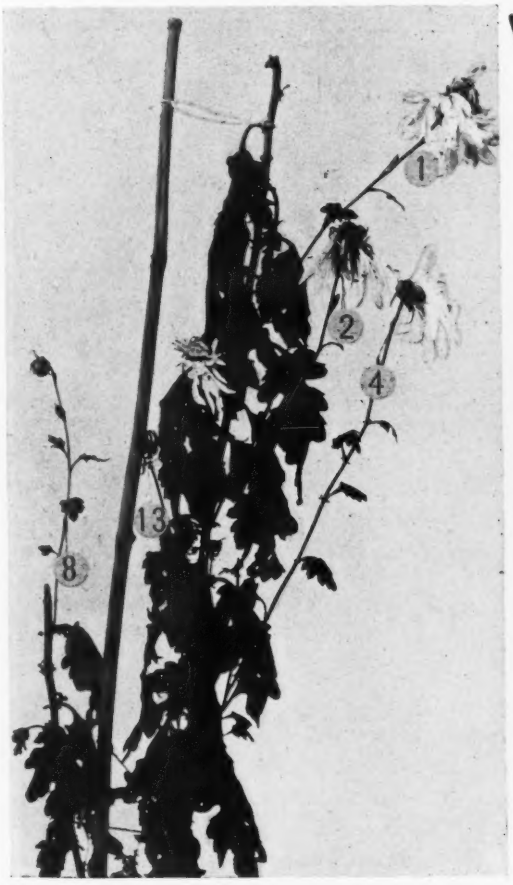


FIG. 14 FIG. 15

FIG. 14. Plant bearing flowers in condition of disease; produced by inoculation. *1*, The topmost head inoculated between the flowers with pycnidia from agar. *2*, The second tallest head as in no. 1. *4*, Inoculated on scarified bark immediately below the head. Dates as above. *13*, Inoculated no. 29 inside involucre, covered by tube 24 hours; diseased December 8. *8*, Inoculated November 24 with agar on scarified bark under wet cotton; diseased December 10.—FIG. 15. Showing method of covering inoculated heads to supply humid atmosphere.

*Series III*

Inoculated November 29, 1906.

*Inoculation no. 22:* agar bearing mycelium was placed between the rays of a head and the whole covered with a tube and supplied with wet cotton to prevent evaporation; tube and cotton removed next day; examined December 8, typical disease.

*Inoculation no. 23:* similar to no. 22 but inoculation made just inside the involucre; examined December 8, typical disease.

*Inoculation no. 24:* similar to no. 22 but upon the tip of the bud; covering tube removed after two days; examined December 8, typical disease.

*Inoculation no. 25:* a single ray from a diseased flower placed among the rays of a flower just beginning to open, which were left uncovered; examined December 8, typical disease.

*Inoculation no. 26:* similar to no. 25; but covered with tube as in no. 22; examined December 8, typical disease.

*Inoculation no. 27:* similar to no. 22; examined December 8, typical disease.

From inoculations nos. 22, 23, 24, and 27 it seems evident the protection of the inoculations from evaporation for 24 or 48 hours leads to greater certainty and rapidity of infection. Inoculations nos. 25 and 26 go to show the lack of necessity for any covering when diseased rays are used with which to inoculate. It is possible that the mycelium is in a more vigorous condition in such diseased rays.

*Series IV*

Inoculations made November 30, 1906, from very vigorously growing mycelium from a plate of cow pea agar.

*Inoculations 28 to 31:* made from the extreme tip of the mycelium taken from the edge of the colony; inoculation under the outer rays; examined December 8, no growth; December 18, typical disease in all.

*Inoculations 32 to 35:* inoculating material taken from the youngest dense zone in the colony; inoculated as in nos. 28 to 31; examined December 8, no growth; December 18, all typically diseased.

*Inoculations 36 to 40:* inoculating material taken from the center

of the colony, the agar being full of old, dark, thick hyphae; examined December 8, all showed typical cases of disease.

It seems apparent from this series that the mycelium from the older central portions is capable of producing quicker infection than that from the outer region of the colony.

## RECOVERY OF FUNGUS FROM INOCULATED HEADS

In many cases rays were taken from the heads which were made diseased by inoculation and these rays were thrown upon Petri dishes containing solidified pea agar, with the result that in each case the petal was soon the center of the pure colony of a fungus identical with that used in the inoculation.

As a result of all of these inoculations it seems clear that the fungus in question is actually the cause of the diseased condition of the blossom; that it can gain entrance to the tissue of the blossom much more easily than to the tissue of the leaves, petioles, and stems; and that humidity at the time of inoculation favors the success of the inoculation.

I desire to acknowledge my indebtedness to Mr. J. G. HALL, Assistant Pathologist, for the illustrations accompanying this article.

## MEDIA USED

*Cow pea agar:* 500 grams of fresh cow pea pods and contents were stewed in one liter of water for one half-hour, drained, 1 per cent. agar added, autoclaved at 110°, and filtered.

*Cow pea leaf agar:* 500 grams of cow pea leaves and petioles were stewed in one liter of water for one half-hour, drained, 1 per cent. agar added, autoclaved at 110°, and filtered.

*Pea agar:* ten garden peas were autoclaved with 1 liter of water for one-half hour, drained, 1 per cent. agar added, autoclaved at 110°, and filtered.

*Four per cent. pea agar:* four per cent. by weight of dry peas were stewed with 1 liter of water for one-half hour, drained, 1 per cent. agar added, autoclaved at 110°, and filtered.

*Standard beef agar:* according to the rules of the American Public Health Association.

*Standard beef gelatin:* according to the rules of the American Public Health Association.

*Pure agar:* 1 per cent. agar without addition of nutrients.

*Litmus lactose agar:* standard beef agar with the addition of 1 per cent. lactose, and litmus sufficient to color.

*Lettuce agar:* 10 grams of lettuce leaves and petioles were stewed in water for one half-hour, drained, 1 per cent. agar added, autoclaved at 110°, and filtered.

*Boiled rice:* 2 grams of washed rice to 8$^{cc}$ of water, tubed, autoclaved at 110°.

*Boiled corn:* 2 grams of washed corn to 8$^{cc}$ of water, tubed, autoclaved at 110°.

*Glucose agar* and *starch agar:* made by adding percentages indicated to pure agar, and sterilizing intermittently.

*Peptone agar:* made by adding per cent. indicated to pure agar and autoclaving at 110°.

WEST RALEIGH, N. C.

# ON THE IMPORTANCE OF PHYSIOLOGICALLY BALANCED SOLUTIONS FOR PLANTS

## II. FRESH-WATER AND TERRESTRIAL PLANTS

W. J. V. OSTERHOUT

(WITH SEVEN FIGURES)

If the facts set forth in the first part[1] of this paper prove to be valid, not for marine plants only, but also for all other kinds, we cannot suppose them to be merely the result of adaptation to a particular environment, but must consider them to be the direct expression of certain fundamental characteristics of living matter. In order that the evidence on this important point might be as complete as possible, a wide range of material was studied. It includes both lower and higher algae, liverworts, Equisetaceae, and several species of flowering plants, embracing among the latter both fresh-water aquatics and land plants. The solutions were made up with all the precautions regarding distilled water and purity of salts described in the first part of this paper. The solutions had the same compositions as there described, except that lower concentrations were employed. A control series was always made, in which all solutions were made faintly alkaline. The material was always rinsed in distilled water before being placed in the solutions. The plants were in all cases exposed to fairly bright light, but not to direct sunlight. The temperature averaged between 18° and 20°C., and was not subject to much fluctuation.

### ALGAE

The most extensive series of experiments on algae was made with Vaucheria and Spirogyra. A form of *Vaucheria sessilis*, abundant in running water, was chosen because it readily gives off zoospores when brought into the laboratory. Tufts of this material, washed free from all adhering dirt, were placed in glass dishes and covered with tap water. Glass slides were placed upright in the dishes. On the following morning numerous zoospores were found

[1] BOTANICAL GAZETTE 42:127–134. 1906.

attached to each slide at the water level. As many as fifty to a hundred zoospores were commonly found arranged in a row across the slide, so that subsequent observation was an easy matter. The slides were thoroughly rinsed in distilled water and transferred to the solutions. The solutions were contained in glass tumblers, in which the slides were placed in an upright position, care being taken to have the zoospores always at the same depth below the surface. The volume of the solution, 100$^{cc}$, was very large compared with that of the zoospores. The tumblers were covered with glass plates to exclude dust and hinder evaporation. Under these circumstances the plants thrive excellently and in favorable solutions produce normal mature fruit.[2]

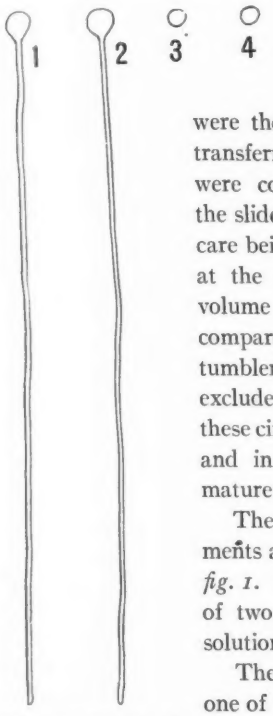



FIG. 1. — Growth of zoospores of Vaucheria during 25 days in various $m/100$ solutions. The quantities are stated in cubic centimeters, the length in millimeters, and the gain in length in per cent. 1, distilled water, length 9.4, gain 5000. 2, NaCl 1000 + $CaCl_2$ 10, length 9.4, gain 5000. 3, NaCl, length 0.18, gain 0. 4, $CaCl_2$, length 0.18, gain 0.

The average results of six series of experiments are given in Table III and illustrated in *fig. 1*. The figure shows clearly how a mixture of two poisonous substances may produce a solution as harmless as distilled water.

The species of Spirogyra employed is a large one of the *majuscula* type. The material was transferred from the pond directly to distilled water; after being rinsed in this it was placed in covered glass dishes containing each 200$^{cc}$ of solution.

It will be seen that these results agree in the most striking way with those already described for marine plants.

Further experiments were made with a variety of other algae, including a species of blue-green alga (Oscillatoria), Chlamydomonas, Closterium and two other species of desmids,

[2] *Cf.* OSTERHOUT, Extreme toxicity of sodium chloride and its prevention by other salts. Jour. Biol. Chemistry 1:363. 1906.

a diatom (Navicula), and a species of Oedogonium. The results agree closely with those given below. There can be little doubt, therefore, that the algae in general, both fresh-water and marine, obey the same law.

TABLE III. ALGAE

A plus sign indicates that the plants were alive when the experiment was stopped. All quantities given are cubic centimeters of $3m/32$ solutions.

| Culture Solution | Duration of Life in Days | |
|---|---|---|
| | Vaucheria | Spirogyra |
| Dilute sea water (total salts 0.6 per cent.) | 40+ | 95+ |
| Dilute artificial sea water: 1000 NaCl, 78 $MgCl_2$, 38 $MgSO_4$, 22 KCl, 10 $CaCl_2$ | 40+ | 95+ |
| Distilled water | 40+ | 95+ |
| Tap water | 40+ | 95+ |
| NaCl | $\frac{1}{100}$ | $\frac{3}{4}$ |
| 1000 NaCl, 10 $CaCl_2$ | 21 | 60 |
| 1000 NaCl, 22 KCl, 10 $CaCl_2$ | 40+ | 65 |
| 1000 NaCl, 78 $MgCl_2$, 10 $CaCl_2$ | 40+ | 65 |
| 1000 NaCl, 78 $MgCl_2$, 22 KCl | $\frac{2}{3}$ | 2 |
| 1000 NaCl, 22 KCl | $\frac{1}{3}$ | 2 |
| 1000 NaCl, 78 $MgCl_2$ | $\frac{2}{3}$ | $1\frac{3}{4}$ |
| 1000 Dist. $H_2O$, 78 $MgCl_2$ = $m/147$ $MgCl_2$ | $1\frac{1}{2}$ | 2 |
| 1000 Dist. $H_2O$, 22 KCl = $m/495$ KCl | $2\frac{1}{2}$ | 3 |
| 1000 Dist. $H_2O$, 10 $CaCl_2$ = $m/1077$ $CaCl_2$ | 9 | 95+ |

LIVERWORTS

The gemmae of Lunularia furnish ideal material for studies on the effects of solutions. They can be obtained at any time of year, they grow readily when floating on the surfaces of solutions, and are fairly uniform in behavior. The material used in these experi-

ments was obtained in part from a greenhouse and in part from moist banks of earth along streams. Material from different sources was never mixed together in any series of experiments.

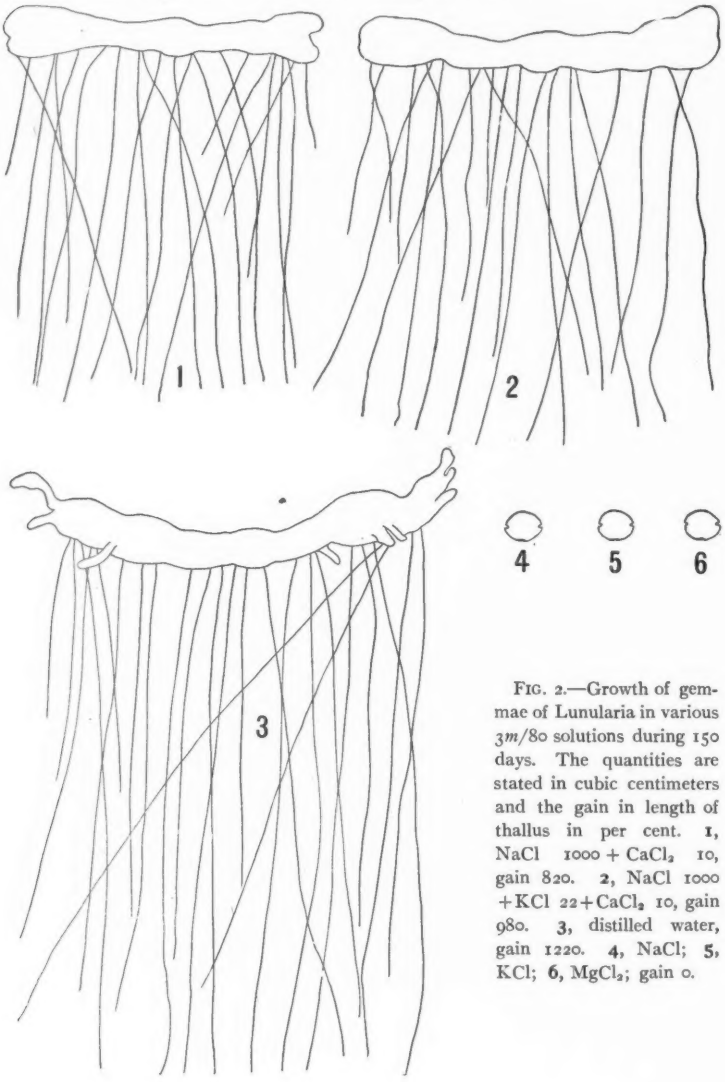



FIG. 2.—Growth of gemmae of Lunularia in various $3m/80$ solutions during 150 days. The quantities are stated in cubic centimeters and the gain in length of thallus in per cent. **1**, NaCl 1000 + $CaCl_2$ 10, gain 820. **2**, NaCl 1000 + KCl 22 + $CaCl_2$ 10, gain 980. **3**, distilled water, gain 1220. **4**, NaCl; **5**, KCl; **6**, $MgCl_2$; gain 0.

The gemmae were removed from the cups with the point of a knife and sprinkled on the surface of the solution, care being taken to exclude particles of dust and dirt. Each tumbler contained 200$^{cc}$ of solution and was covered with a glass plate. The gemmae may be easily removed from the surface of the solution for purposes of observation by dipping into it a clean slide and slowly raising it at an angle so as to take up the material upon one side only. The material should not be replaced in the solution unless extreme care be taken to prevent contamination.

The average results of six series of experiments are given in Tables IV and V, and illustrated in *figs. 2* and *3*.

TABLE IV. LUNULARIA

A plus sign indicates that the plants were alive when the experiment was stopped. All quantities given are cubic centimeters of $3m/32$ solutions.

| Culture Solution | Duration of Life in Days |
|---|---|
| Dilute sea water; total salts 0.6 per cent..... | 200+ |
| Dilute artificial sea water: 1000 NaCl, 78 $MgCl_2$, 38 $MgSO_4$, 22 KCl, 10 $CaCl_2$ ..... | 200+ |
| Distilled water..... | 200+ |
| NaCl..... | 4 |
| 1000 NaCl, 10 $CaCl_2$ ..... | 100 |
| 1000 NaCl, 22 KCl, 10 $CaCl_2$ ..... | 200+ |
| 1000 NaCl, 78 $MgCl_2$, 10 $CaCl_2$ ..... | 200+ |
| 1000 NaCl, 78 $MgCl_2$, 22 KCl ..... | 9 |
| 1000 NaCl, 22 KCl ..... | 4 |
| 1000 NaCl, 78 $MgCl_2$ ..... | 7 |
| $MgCl_2$..... | 2 |
| KCl ..... | 12 |
| $CaCl_2$..... | 100 |

TABLE V. LUNULARIA

All quantities given are cubic centimeters of $3m/80$ solutions

| Culture Solution | Growth in 150 Days | | |
|---|---|---|---|
| | Length of thallus in mm. | Per cent. increase in length of thallus | Aggregate length of rhizoids per thallus in mm. |
| Dilute artificial sea water: 1000 Nacl, 78 $MgCl_2$, 38 $MgSO_4$, 22 KCl, 10 $CaCl_2$ | 6.52 | 1204 | 168 |
| Distilled water | 6.60 | 1220 | 180 |
| NaCl | 0.50 | 0 | 0 |
| 1000 NaCl, 10 $CaCl_2$ | 4.60 | 820 | 114 |
| 1000 NaCl, 22 KCl, 10 $CaCl_2$ | 5.40 | 980 | 123.5 |
| 1000 NaCl, 78 $MgCl_2$, 10 $CaCl_2$ | 5.50 | 1000 | 125 |
| 1000 NaCl, 78 $MgCl_2$, 22 KCl | 0.54 | 8 | 0.3 |
| 1000 NaCl, 22 KCl | 0.50 | 0 | 0 |
| 1000 NaCl, 78 $MgCl_2$ | 0.52 | 4 | 0.1 |
| $MgCl_2$ | 0.50 | 0 | 0 |
| KCl | 0.50 | 0 | 0 |
| $CaCl_2$ | 4.55 | 810 | 90 |

### EQUISETUM

The spores of Equisetum retain their vitality for only a few days. The fruiting cones were brought into the laboratory and allowed to stand for a day or two. The freshly shed spores were then placed on the surfaces of solutions in covered glass dishes. They germinate rapidly and in a few days produce prothallia of fair size. The average results of four series of experiments are shown in Table VI and *fig. 4*.

### FLOWERING PLANTS

The most extensive series of experiments was made with wheat. The variety selected is known as Early Genesee. The percentage

of germination is very high and the growth is vigorous from the start. The plan first tried was that of carefully placing the seeds on the surface of the solutions so that they float. This worked well with

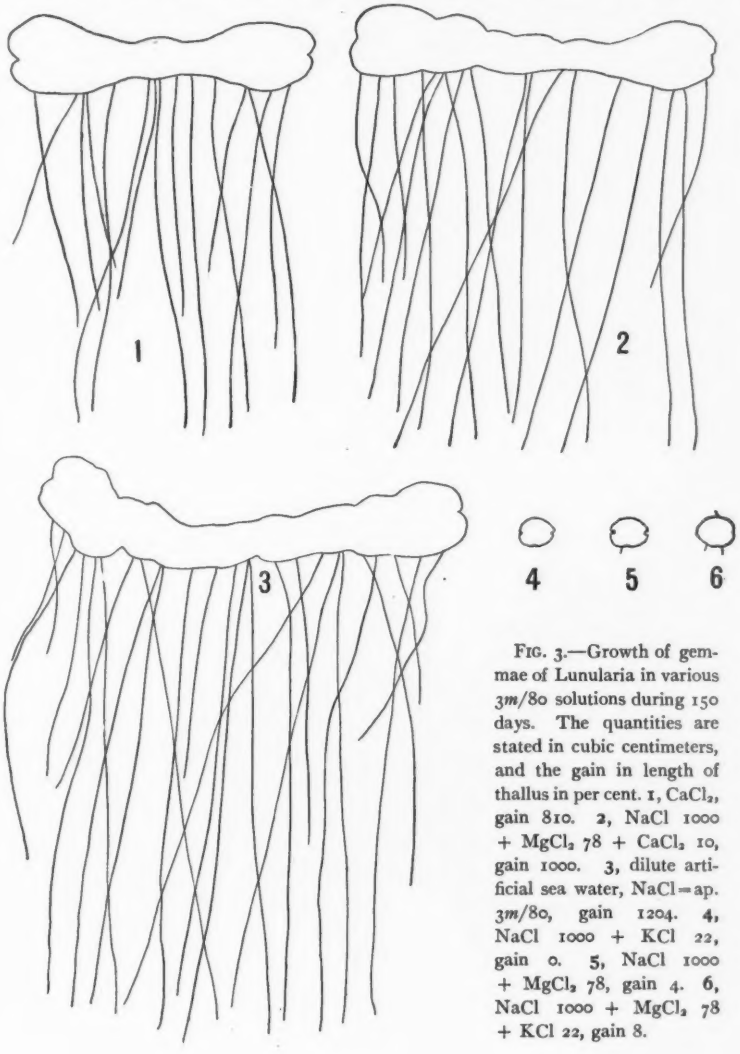



FIG. 3.—Growth of gemmae of Lunularia in various $3m/80$ solutions during 150 days. The quantities are stated in cubic centimeters, and the gain in length of thallus in per cent. 1, $CaCl_2$, gain 810. 2, NaCl 1000 + $MgCl_2$ 78 + $CaCl_2$ 10, gain 1000. 3, dilute artificial sea water, NaCl = ap. $3m/80$, gain 1204. 4, NaCl 1000 + KCl 22, gain 0. 5, NaCl 1000 + $MgCl_2$ 78, gain 4. 6, NaCl 1000 + $MgCl_2$ 78 + KCl 22, gain 8.

wheat and other small seeds during the first stages of germination; but if the experiments are to be carried beyond this stage, the seedlings must be supported so that the leaves do not come into contact with the solution. After some trials the following device was hit upon which answers the purpose admirably. A strip of filter paper is folded lengthwise and one of the folds turned back as shown in *fig. 5*. The seeds are placed in the trough thus formed and the whole strip is then bent into a circle and placed in a tumbler previously filled with solution. The strip should be of such length that when placed in the top of the tumbler the ends just meet and so form a stiff collar which just fits inside the top of the tumblers and which will not slip down. A large number of these collars may be prepared, filled with seeds, bent into circles, and secured by ordinary paper-clips placed on the overlapping ends. They may be piled in trays until wanted. It is then only necessary to remove the clips and set the collars in glasses previously filled with solutions.

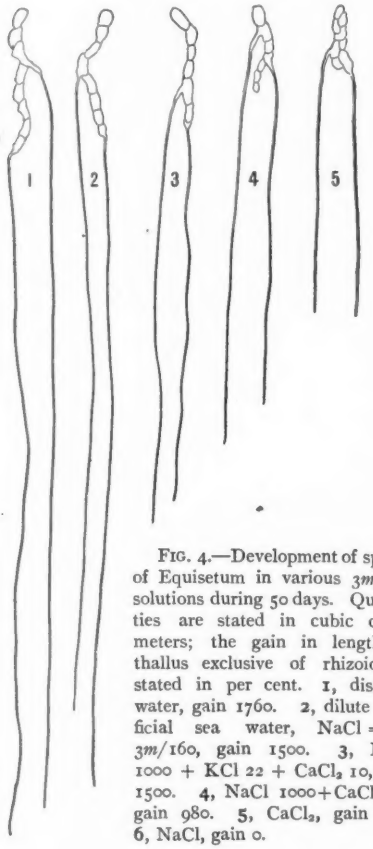



FIG. 4.—Development of spores of Equisetum in various $3m/160$ solutions during 50 days. Quantities are stated in cubic centimeters; the gain in length of thallus exclusive of rhizoids is stated in per cent. **1**, distilled water, gain 1760. **2**, dilute artificial sea water, NaCl = ap. $3m/160$, gain 1500. **3**, NaCl 1000 + KCl 22 + $CaCl_2$ 10, gain 1500. **4**, NaCl 1000 + $CaCl_2$ 10, gain 980. **5**, $CaCl_2$, gain 700. **6**, NaCl, gain 0.

In some cases, especially where larger glasses are employed, a strip of paper of double thickness may be used; this makes a stiffer

collar. It is then advisable to perforate the bottom of the seed trough by means of a tracing wheel such as is used for patterns. This allows the roots to penetrate the paper freely and without delay.

Care should be taken that the solution does not cover the seeds. The paper must be spread open at the top so as to allow the air to come into direct contact with the seeds. The micropyle should be in contact with the moist filter paper.

Careful experiments were made to determine whether the filter paper exerted any influence on the solution (by absorption, etc., or by concentration of the solution about the seed as the result of evaporation) which might affect the results, but no such influence

TABLE VI. EQUISETUM

All quantities given are cubic centimeters of $3m/160$ solutions.

| Culture Solution | Growth in 50 Days | | |
|---|---|---|---|
| | Length of thallus in mm. | Per cent. increase in length of thallus, exclusive of rhizoids | Aggregate length of rhizoids per thallus in mm. |
| Dilute artificial sea water: 1000 NaCl, 78 $MgCl_2$, 38 $MgSO_4$, 22 KCl, 10 $CaCl_2$ | 0.80 | 1500 | 8.1 |
| Distilled water | 0.93 | 1760 | 9.0 |
| NaCl | 0.05 | 0 | 0 |
| 1000 NaCl, 10 $CaCl_2$ | 0.54 | 980 | 4.7 |
| 1000 NaCl, 22 KCl, 10 $CaCl_2$ | 0.80 | 1500 | 5.2 |
| 1000 NaCl, 78 $MgCl_2$, 10 $CaCl_2$ | 0.93 | 1760 | 9.0 |
| 1000 NaCl, 78 $MgCl_2$, 22 KCl | 0.07 | 40 | 0 |
| 1000 NaCl, 22 KCl | 0.05 | 0 | 0 |
| 1000 NaCl, 78 $MgCl_2$ | 0.07 | 40 | 0 |
| $MgCl_2$ | 0.05 | 0 | 0 |
| KCl | 0.05 | 0 | 0 |
| $CaCl_2$ | 0.40 | 700 | 3.2 |

could be detected. The solutions were renewed from time to time and the concentration ascertained by occasional titration.

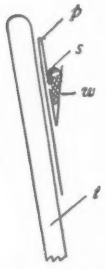



FIG. 5.—Sectional view of wall of tumbler and seed supported by folded filter paper; *p*, paper; *s*, seed; *t*, tumbler; *w*, water line.

It should be said that in general the growth of roots (or any parts in direct contact with the solution) furnishes a much better criterion of the effect of solutions than the aerial portions of the plant. In certain solutions which are so poisonous that the roots cannot develop, the leaves may grow fairly well for a time. In these cases the poisonous solutes are apparently filtered out by the tissues of the seed as the solution passes through them on its way to the leaf. For this reason the figures for the growth of roots only are here given. The results are shown in Table VII and *figs. 6* and *7*, which give the average of five series of experiments. Each number represents average measurements of at least four or five hundred seeds. This is necessary in order to do away with the individual variation so common in seeds.

TABLE VII. WHEAT

All quantities given are cubic centimeters of $3m/25$ solutions.

| CULTURE SOLUTION | GROWTH IN 40 DAYS: Aggregate length of roots per plant in mm. |
|---|---|
| Dilute artificial sea water: 1000 NaCl, 78 $MgCl_2$, 38 $MgSO_4$, 22 KCl, 10 $CaCl_2$ | 360 |
| Distilled water | 740 |
| NaCl | 59 |
| 1000 NaCl, 10 $CaCl_2$ | 254 |
| 1000 NaCl, 22 KCl, 10 $CaCl_2$ | 324 |
| 1000 NaCl, 78 $MgCl_2$, 10 $CaCl_2$ | 327 |
| $MgCl_2$ | 7 |
| KCl | 68 |
| $CaCl_2$ | 70 |

A similar though less extensive series of experiments was carried out with flax, alfalfa, red-beet, and radish seeds. Another series was made by placing pieces of the fresh-water aquatics, Zannichellia and Potamogeton, in solutions, or in the case of Lemna, by allowing the plants to float on the surface. The results in all these cases were similar to those given above.

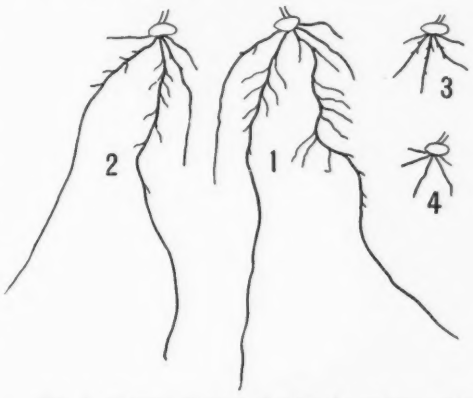



FIG. 6.—Growth of roots of wheat in various $3m/25$ solutions. Quantities are stated in cubic centimeters, and the aggregate length of roots in millimeters. **1**, NaCl 1000 + KCl 22 + $CaCl_2$ 10, length 324. **2**, NaCl 1000 + $CaCl_2$ 10, length 254. **3**, $CaCl_2$, length 70. **4**, NaCl, length 59.

It is thought desirable to see how cuttings would behave under similar treatment. Cuttings (about nine inches in length) of Tradescantia and Tropaeolum were placed upright in bottles, the lower three inches of the plant being submerged

TABLE VIII. CUTTINGS

All quantities given are cubic centimeters of $3m/32$ solutions.

| Culture Solution | Growth in 10 Days | |
|---|---|---|
| | Tradescantia | Tropaeolum |
| Dilute artificial sea water: 1000 NaCl, 78 $MgCl_2$, 38 $MgSO_4$, 22 KCl, 10 $CaCl_2$ | Long roots | Long roots |
| Distilled water | Very long roots | Very long roots |
| NaCl | No roots | No roots |
| 1000 NaCl, 10 $CaCl_2$ | Short roots | Short roots |
| 1000 NaCl, 22 KCl, 10 $CaCl_2$ | Medium length roots | Medium length roots |

in the solution. Absorbent cotton was packed in the neck of the bottle to exclude dirt and hinder evaporation. The results were similar to those described above, as will be seen from Table VIII.

Finally the question was raised whether the tissues of the stem and leaf, if brought into direct contact with the solution, would behave like the root. To answer this, sections of considerable (but uniform) thickness were cut with a microtome and placed in the solutions. The results appear in Table IX.

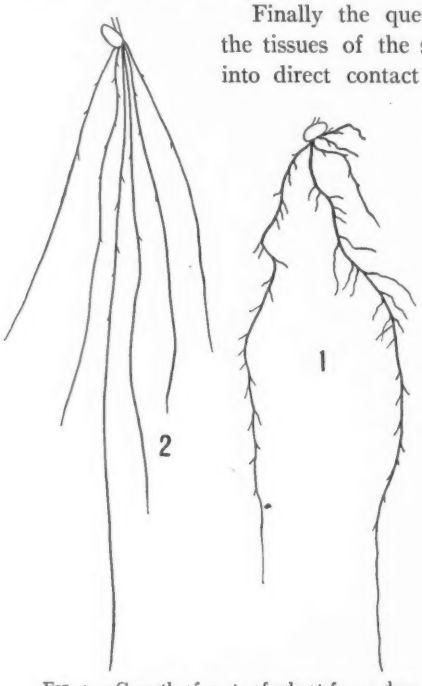



FIG. 7.—Growth of roots of wheat for 40 days. 1, in dilute artificial sea water (NaCl = ap. $3m/25$), aggregate length of roots 360mm. 2, in distilled water, aggregate length of roots 740mm.

The results described in this paper are in all essentials in striking agreement with those obtained from the study of marine plants, as well as from the study of marine and fresh-water animals as referred to in the first part of this paper. This agreement shows that the principle of balanced solutions is of general validity.[3] The application of this principle to soil and river water[4] and to nutrient solutions, I hope to take up in a subsequent paper.

[3] LOEW and his pupils have shown that calcium antagonizes magnesium (cf. Bull. No. 18, Div. Veg. Phys. and Path. U. S. Dept. Agric. 1899). See also the antagonistic effects noted by KEARNEY and CAMERON (Report No. 71, U. S. Dept. Agric. 1902) in their studies on the salts of alkali soils. The method employed by them (observation of the root-tip only) is so different from mine that I have not attempted to compare the results.

[4] In the first part of this paper I have referred to the composition of tap water, but it seems advisable to defer the discussion of this point.

TABLE IX. SECTIONS

All quantities given are cubic centimeters of $3m/32$ solutions.

| Culture Solution | Duration of Life in Days | | | |
|---|---|---|---|---|
| | Red beet: Cross-sections of root | Mesembry-anthemum: Cross-sections of leaf | Tradescantia: Cross-sections of stem | Tropaeolum: Cross-sections of leaf |
| Dilute artificial sea water: 1000 NaCl, 78 $MgCl_2$, 38 $MgSO_4$, 22 KCl, 10 $CaCl_2$ | 35 | 16 | 19 | 25 |
| Distilled water | 35 | 18 | 20 | 32 |
| NaCl | 18 | 9 | 12 | 14 |
| 1000 NaCl, 10 $CaCl_2$ | 25 | 12 | 18 | 20 |
| 1000 NaCl, 22 KCl, 10 $CaCl_2$ | 30 | 15 | 19 | 22 |

For the sake of clearness it seems desirable to call attention to the distinction between balanced solutions and ordinary nutrient solutions. A nutrient solution may be used in such dilute form that none of its components could exert any toxic action even if the other constituents were removed. In this case there are no poisonous effects to be inhibited and consequently no balancing of the solution is required. Our only concern is to supply all the substances needed for nutrition, irrespective of any balancing action, and so form a *complete* nutrient solution.

If we increase the concentration of this solution, however, we soon reach the point where some or all of the components begin to exert their individual toxic effects, whereupon it may become necessary to inhibit these effects by proper adjustment of the relative proportions of the substances present or by the addition of other substances. The substances added to produce a balance do not necessarily have a nutritive value. For example, Loeb[5] was able to balance certain solutions by adding zinc, cobalt, aluminum, etc.[6]

[5] Am. Jour. Physiology **6**:411–433. 1902.

[6] To make clear this distinction between balanced and nutrient solutions is more necessary, since Loew and Aso (Bull. Coll. Agr. Tokyo Imp. University **7**:395. 1907) confuse the two kinds of solutions. Their criticisms are wholly based on this misconception and do not affect the matter as I have presented it. The distinction between nutrient and balanced solutions is due to Loeb, who has explained it clearly in his *Dynamik der Lebenserscheinungen* 115–120.

In general we may know when the solution is properly balanced by comparing its effects with those of *pure*[7] distilled water. In a properly balanced solution we expect the organism to live approximately as long as in distilled water, and while it will not grow so fast (on account of the osmotic pressure), the ultimate development reached should be comparable with that attained in distilled water.[8]

Why all these effects are so, we are not at present prepared to say in detail. LOEB has gone farther than any other in the explanation of these phenomena, referring them to the effects of salts and ions on proteids[9]. According to his conception any metal must be poisonous when it alone is present in the solution, for it will enter the proteids and drive out other metals in accordance with the law of mass action. This will of course alter the properties of the proteids and so cause disturbances in function. The only way to prevent this is to maintain a proper balance between the various metals in the solution. It may be pointed out that an analogy exists between the effects described here and various reactions in which proteids are involved. Antagonism between Na and Ca, for example, is see in the clotting of blood, which is hindered by Na and favored by Ca.

The thing of chief importance is the agreement in behavior of such a great diversity of plants with the fresh-water and marine animals already studied. Thereby is brought to light a new point of similarity between animals and plants which is fundamental in character and which must be taken into consideration in attempting to formulate a theory of living matter.

UNIVERSITY OF CALIFORNIA

[7] Water twice distilled from glass, the first third of the distillate being rejected, is usually regarded as pure. But such water may be quite poisonous if any part of the apparatus, including stoppers, be new. The longer the apparatus is used the less poisonous the water becomes, until it finally ceases to be toxic.

[8] Higher concentrations excepted.

[9] See references in the first part of this paper, BOT. GAZ. **42**: 134. 1906

# FERTILIZATION AND EMBRYOGENY IN EPHEDRA TRIFURCA[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 102

W. J. G. LAND

(WITH PLATES XX–XXII)

Little attention has been given to the fertilization and embryogeny of Ephedra, the only accounts being those of STRASBURGER and JACCARD. In 1872 STRASBURGER,[2] studying *E. altissima*, described and figured unfertilized eggs and four stages of the embryo immediately before and after formation of cotyledons. In 1876 he described[3] a series of stages in *E. altissima* from the central cell of the archegonium to the formation of suspensors. He noted the penetration of the pollen tube, which, he says, reaches the egg through a canal formed by the breaking-down of the neck cells of the archegonium. Fertilization was not observed, but several stages of the proembryo were noted. Usually three to eight proembryos are formed and arranged in a row down the middle of the egg. He calls attention to what he considers an abnormal condition, in which the cytoplasm of the egg is pierced through by irregular tortuous channels supported throughout their length by kinoplasmic fibers. These channels extend around and finally inclose a portion of the egg cytoplasm together with a single nucleus. He thinks these channels result from the activities of this single nucleus which is finally inclosed. The breaking-out of the suspensors from the archegonia was noted. His third account,[4] published in 1879, deals at some length with the embryogeny of *E. campylopoda*, but adds little to the results obtained from *E. altissima*.

In 1894 JACCARD,[5] studying *E. helvetica*, observed the pollen tube

[1] Read before the Botanical Society of America, December 1906; abstract published in Science N. S. **25**: 282, 283. 1907.

[2] STRASBURGER, E., Die Coniferen und die Gnetaceen. 1872.

[3] STRASBURGER, E., Ueber Zellbildung und Zellteilung. 1876.

[4] STRASBURGER, E., Die Angiospermen und die Gymnospermen. 1879.

[5] JACCARD, P., Recherches embryologiques sur l'*Ephedra helvetica*. Inaugural Dissertation. Lausanne. 1894.

entering the archegonium, one male nucleus in contact with the egg nucleus, and the second male nucleus, the stalk cell, and the tube nucleus lying in the micropylar end of the egg. He also studied pollen tubes grown in five per cent. gelatin in sterilized pear juice. He noted the disorganization of the jacket cells and describes a fusion of the nuclei of the jacket cells with one another and their subsequent migration into the cytoplasm of the egg. He also observed a large number of small nuclei in the egg, the nature and origin of which he was not able to determine. He further states that a suspensor ("prosuspenseur" or "embryonalschlauch") is not formed. The elongation of the basal cells of the embryo to form a secondary suspensor ("le columelle") is noted.

The present study of the fertilization and embryogeny of *E. trifurca* is the continuation of the paper[6] which dealt with spermatogenesis and oogenesis in the same species. Material for the study was collected at Mesilla, New Mexico, chiefly in April 1904. Much of the material collected in 1903 was unfit for a study of fertilization stages because of the ravages of a parasitic insect which attacked the ovules about the time of pollination. Embryos having been observed about April 20, 1903, it was hoped that by beginning to make collections as early as April 1 in the following season and continuing them at intervals of two days a fairly complete series could be obtained. Collecting was discontinued April 20, 1904, at which time the majority of embryos had attained their maximum size. The season of 1904 was unprecedentedly hot and dry and in consequence development was much accelerated, so much so in fact that embryos with large secondary suspensors were found in the material collected April 1. Not all of the material collected at the earliest date had been pollinated. In 1905 staminate strobili sent from the Carnegie Desert Laboratory, Tucson, Arizona, made possible the final study of the male gametophyte.

## POLLEN TUBES AND FERTILIZATION

Pollen grains were placed in a ten per cent. solution of saccharose on a slide in a moist chamber. By evaporation the solution became considerably denser toward the close of the experiment. Shortly

[6] LAND, W. J. G., Spermatogenesis and oogenesis in *Ephedra trifurca*. BOT. GAZETTE 38: 1–18. *pls. 1–5.* 1904.

after being placed in the sugar solution, the fluted exine is ruptured by a longitudinal split extending along one side of the grain from end to end, and the intine with its contents is completely freed. The two prothallial cells soon disappear and the cytoplasm of the prothallial end becomes very vacuolate. The body cell next divides, the spindle being parallel with the long axis of the pollen grain (*fig. 1*). At the same time the tube nucleus and the stalk cell, which lie at opposite ends of the grain, each move through ninety degrees and lie side by side, or occasionally they may lie as shown in *fig. 2*. The tube nucleus comes to rest against the wall, assumes a lenticular shape, and soon afterward the pollen tube is put out from this point of contact. Appearances seem to indicate that the pollen tube is produced at the point of separation of the tetrads, but this could not be definitely determined because of the symmetry of the pollen grain and the possibility of a slight rotation of the extruded mass after escaping from the exine.

The male nuclei are of equal volume and are truly elliptical. One of them almost invariably passes in advance of the stalk cell, which takes a position in advance of the rearmost male nucleus (*fig. 3*). Measurements of male nuclei show them to be exactly of like dimensions; neither is there any optical difference. Their relative position in the pollen tube is the only evidence, if evidence it be, of any inequality. There is little doubt that the male nucleus nearest the end of the tube will succeed in fusing with the egg nucleus because of its favorable position. Within ten hours from the time the pollen grains were placed in the sugar solution the pollen tubes were sufficiently long to reach the egg. After twenty-four hours the tubes ceased to elongate, at which time they were three to four times longer than is necessary to penetrate to the egg. The stalk nucleus is usually about half the diameter of the male nucleus. The tube nucleus is variable in form, as is common among tube nuclei of gymnosperms.

As has been shown in the study of spermatogenesis and oogenesis, the pollen grains come to rest on the bottom of the pollen chamber in contact with the gametophyte, in the vicinity of or on the necks of the archegonia. Occasionally pollen grains rest on the sides of the pollen chamber as well as on the bottom, but by far the greater number rest on the bottom, and in consequence the pollen tubes are put out directly into the archegonia. Since all material studied was fixed

at least four days after pollination, and fertilization is possible within ten hours after pollination, it has been very difficult to secure fertilization and early proembryonal stages. Only those ovules which have been incompletely pollinated by having the pollen lodged for a time in the long tubular micropyle, and later dislodged and reaching the pollen chamber, can by any possibility show fertilization. From the nature of things such a delay in pollination must be very rare, although the ovules are peculiarly fitted to have such delays occur. Among over 2800 ovules sectioned at the time when fertilization was expected, only two (*figs. 4* and *5*) showed early stages.

The pollen tube forces its way between the neck cells of the archegonium, rarely destroying them in its passage. Only in one or two instances were the lower neck cells destroyed. In *fig. 4* the lower and apparently double nucleus shows the all but complete fusion of the sperm and the egg nuclei. The small nucleus lying immediately above the fusing nuclei is the ventral nucleus; the one immediately above it is the second male nucleus, which in this instance has passed deeper into the egg cytoplasm than usual. *Fig. 5* shows the fusion nucleus with fragments of the ventral nucleus lying above it. The second male nucleus, with the stalk nucleus and tube nucleus lying immediately below it, is seen in the upper end of the egg. The stalk nucleus and the tube nucleus soon disappear. The second male nucleus does not ordinarily penetrate deeper into the egg than is shown in *fig. 5*, but undergoes its final changes here. The dense mass of cytoplasm surrounding the fusion nucleus and extending downward through the egg is well shown in *figs. 4* and *5*. This mass first appears as a densely staining spherical body in the upper third of the central cell, and disappears with the formation of proembryos.

## EMBRYOGENY

The fusion nucleus gives rise to eight free nuclei, more or less unequal in size, and three to five of the largest ones surround themselves by a wall and produce embryos. The free nuclei are either arranged in a row down the middle of the egg, or more frequently are scattered through it. The lower ones in general successfully produce embryos, although a few instances were noted in which the lower ones did not function further than the one-celled stage, the micropylar ones

alone being successful. Nearly all of the largest vacuoles in the egg disappear shortly after fertilization, and the cytoplasm becomes more and more homogeneous as free nuclear division progresses. The proembryonal nuclei usually become so dense that they can be seen with difficulty in the cytoplasm.

Delicate cytoplasmic radiations, similar to those figured in the unfertilized egg, stream out from each nucleus and end in a denser ring of cytoplasm. This delicately radiating cytoplasm becomes more and more vacuolate, finally taking on the appearance shown in *figs. 6* and *7*. In *fig. 6* the large cell is one of two proembryonal cells which later will produce suspensors. *Fig. 7* shows two or four functioning proembryos. The first suggestion of walls, or rather cytoplasmic thickenings, is shown in *figs. 6* and *7*. The functioning proembryos isolate themselves from the surrounding cytoplasm as follows. Cleavage cracks appear, starting presumably at the equatorial region of the last spindle, although all trace of a spindle has disappeared before cleavage is apparent. An early stage of incomplete cleavage between sister cells is shown in *fig. 8*. The cleavage cracks, following the feebly defined wall or cytoplasmic thickening, continue until they meet around the nucleus, carving out a more or less irregular mass of cytoplasm for each nucleus. Three of five completely isolated proembryos are shown in *fig. 9*, which also shows masses of egg cytoplasm that have not been appropriated by the proembryonal cells.

At the time the proembryonal cells have freed themselves completely from the egg cytoplasm they lie in a nutritive mass derived from various sources. Shortly after fertilization, the walls of the jacket cells, which always have been extremely tenuous, disappear and in most preparations the jacket-cell cytoplasm becomes mixed with that of the egg (*fig. 9*). Shortly before the walls disappear the jacket cells become binucleate, the nuclei dividing either mitotically or amitotically. Occasionally mitotic divisions occur simultaneously in every cell of both layers of the jacket, or every division may be amitotic, or both kinds of division may occur in the same jacket. When the latter occurs, the nuclei at the upper end of the archegonium divide amitotically, and these amitotic divisions can easily be mistaken for fusion. Occasionally the jacket nuclei enlarge, fragment, and their chromatin is scattered through the cytoplasm of the jacket cell and

later passes out into the egg cytoplasm. Sometimes this fragmentation is accompanied by kinoplasmic fibers, as shown in *fig. 10*. *Fig. 9* also shows an intact jacket-cell nucleus which has passed into the egg cytoplasm. Occasionally, though rarely, instances were noted in which the jacket cells do not become binucleate and break down at the time of fertilization; some of them, especially those near the middle of the archegonium, have the nucleus surrounded by delicate cytoplasmic radiations similar to those noted in the unfertilized egg. These nuclei also very much resemble the egg except in size, being of course much smaller. Apogamy was at first suspected, but further study showed that these egglike jacket nuclei do not function, being finally broken down and absorbed by the embryos.

The second male nucleus is normally in the position shown in *fig. 5*. It enlarges and occupies the upper part of the egg and extends into the space recently filled by the adjacent jacket cells. The chromatin is arranged in strands, at the ends of some of which it may be aggregated in small groups (*figs. 6, 7*). The nuclear membrane breaks down on one side (*fig. 6*) and cleavage planes pass up into the nucleus. *Fig. 7* shows a male nucleus with an irregular mass of spindles with groups of chromatin granules at the poles. Later the region occupied by the male nucleus is filled with a number of small cells which may have come from further division of the second male nucleus, or some of them may be a joint product of chromatin from the male nucleus and from the jacket nuclei. Below this region are numerous small cells which possibly may have resulted either from further division of the functionless proembryos, or by division of the jacket nuclei which have wandered into the egg cytoplasm or from both sources, and still others from masses of cytoplasm which may have been separated into smaller masses of cytoplasm by cleavage planes, as shown in *fig. 9*. Numbers of the very small cells seem to have a single granule in the center; others appear to be only masses of cytoplasm. This nutritive mass, presumably derived from so many sources, is very ephemeral and is quickly absorbed by the functioning proembryos.

In the ephemeral nutritive mass in which the proembryos lie it seems that we have at least a suggestion of the origin of endosperm. The term endosperm is here used in the strict angiosperm sense and

must not be confused with the female gametophyte of gymnosperms. Many things lead to the belief that the chromatin of the second male nucleus, together with that of the jacket nuclei, has something to do with the production of at least a part of the mass of small cells which appears in the egg and jacket region. It is at the edge of the disintegrating male nucleus that spindles first appear, with either a few granules or masses of chromatin at their poles. The absolute equality of the male nuclei, for they differ in no visible way except in position in the pollen tube, seems to suggest that the nucleus which does not fuse with the egg nucleus functions in some other way; for cells which cease to function are either eliminated or show signs which point to their future elimination. If it can be shown beyond doubt that the chromatin of the second male nucleus together with some of the jacket-cell nuclei or the chromatin from these nuclei are jointly responsible for some of the small cells in the egg and jacket region, it follows that we have the foreshadowing if not the real beginning of endosperm. At least it may for the present be designated as physiological endosperm. The apparently universal occurrence of triple fusion in angiosperms seems to preclude the possibility that they may furnish a solution for this problem. It is not impossible that the Gnetales, which in so many ways have reached the angiosperm level, may have some member which will give an answer to the problem of the origin of the angiospermic endosperm. The ephemeral mass of nutritive cells is certainly larger in *E. trifurca* than the endosperm mass of many angiosperms.

The functioning proembryonal cells round out as the adjacent nutritive mass is absorbed (*fig. 10*). The nucleus of a proembryonal cell divides, giving rise to two free nuclei, which at first are equal in size, but one soon gains an ascendancy over the other, becoming noticeably larger. A large vacuole develops in the upper part of the cell and the free nuclei pass down and to one side of the cell if it is one of the upper proembryos, but to the bottom if it is the lowest one in the archegonium. The larger nucleus is invariably placed lowest in the cell. Two papillae, seemingly initiated by the nuclei, are put out by the cell wall from the points nearest the nuclei (*fig. 11*). Soon the papilla nearest the larger nucleus rapidly elongates and the other papilla disappears (*fig. 13*). In one instance four free nuclei, one

large, two smaller, and one very minute, were observed in a proembryonal cell. The suspensor tube is immediately directed downward toward the antipodal region if the cell is the lowest one in the archegonium, but outward and then downward if from one of the upper cells. *Fig. 13* shows the suspensor tube before either of the nuclei has entered it. The larger nucleus passes down nearly to the end of the suspensor tube, and immediately behind it a cleavage ring, beginning at the wall of the suspensor tube and gradually extending inward, appears in the cytoplasm and separates it into two masses, each containing a nucleus. *Fig. 14* shows the cleavage ring incomplete; and *fig. 15* is a much higher magnification of the same section. A wall separating the suspensor tube into two regions is then established at the cleavage plane.

The nucleus left behind (*fig. 14*) enlarges, enters the suspensor (*fig. 16*), becomes the suspensor nucleus, passes down, and remains in the lower end of the suspensor (*figs. 17, 18*), where it finally disintegrates (*fig. 19*). The suspensor quickly elongates, thrusts the embryo initial deeply downward into the gametophyte, and remains turgid until the secondary suspensor appears (*fig. 23*), when it collapses. The longest suspensor noted reached a length of 3.1 mm.

The nucleus of the embryo initial cell divides and a transverse wall is laid down in the usual way along the cell plate (*fig. 18*). *Fig. 19* shows a two-celled embryo with the wall fully established. An anticlinal division of the basal cell next occurs, the wall being either parallel with the suspensor (*fig. 21*) or slightly inclined (*fig. 20*). *Fig. 22* is immediately before the differentiation of dermatogen, which is cut off early, as shown by *fig. 23*. The latter figure also shows the first appearance of the secondary suspensor, a later stage of which is shown in *fig. 24*. The secondary suspensor is added to by successive elongations of basal cells of the embryo and finally merges insensibly into the root cap. There are three stages of suspensor formation: the suspensor tube, initiated by the activity of the nucleus which finally becomes the embryo-initial nucleus; the single suspensor, derived from the basal portion of the suspensor tube; and lastly the secondary suspensor ("le columelle" of JACCARD), derived from the basal cells of the embryo.

Coincident with fertilization and development of the proembryos,

the gametophyte is stimulated to further activity. A single plate of the outermost layer of cells of the gametophyte, forming the bottom of the pollen chamber and extending outward a short distance under the tip of the nucellus, becomes meristematic (*fig. 25*), and by repeated periclinal and a few anticlinal divisions forms a plug which effectually closes the pollen chamber and receives the backward thrust of the elongating cells of the gametophyte and later of the embryo; this plug persists until absorbed by the maturing embryo. The antipodal region begins active division and the bulk of the gametophyte is materially increased. The cells near the archegonia first elongate, followed successively by groups of cells extending across the gametophyte. The elongated cells after having lost their contents are folded back and forth in the micropylar region (*fig. 25*), being prevented from escaping through the pollen chamber by the pollen-chamber plug. This elongation of groups of cells, proceeding successively from the micropylar to the antipodal region, serves admirably to keep an abundance of food constantly near the embryo.

As the seed matures the integuments and nucellus become gorged with food. The epidermal cells, especially those of the nucellus, are filled with starch and become so turgid that they are frequently torn loose from the layer beneath (*fig. 26*).

In the collection which reached Chicago on May 11, 1903, numbers of young plants were escaping from strobili still attached to the stem. This shows that under conditions favorable for growth the period of rest of the seed of *E. trifurca* is a brief one, if indeed it has a resting-period.

Staminate and ovulate strobili were first recognized in December, but they had probably been set the preceding month and perhaps earlier. The time from the setting of the strobili to the "germination" of the seed is therefore approximately six months. Compared with pines the time is short, for the latter require about thirty-six months.

## DISCUSSION

The ovulate strobilus of Ephedra is compound, a character common to all Gnetales and shared by the Coniferales. The staminate strobilus, as in other Gnetales, is also compound, a character not found in other gymnosperms. Perhaps the nearest approach to a com-

pound staminate strobilus outside the Gnetales is the short branch of *Torreya taxifolia*,[7] which bears a simple staminate strobilus in the axil of each leaf of the short branch. It is conceivable that by shortening the internodes in such a branch, combined with a further reduction of the sporophyll number, a compound strobilus will result; it is at least suggestive of the way in which staminate and ovulate compound strobili may have originated.

The two integuments of the ovule of Ephedra, a character shared with the Taxineae, may be regarded as a primitive condition, a condition even more primitive than in Taxineae, for the nucellus is free for a considerable part of its length. Also the inner integument first appears as two parts, and the outer as four, each distinct in origin. The tendency in both gymnosperms and angiosperms seems to be toward union of the two integuments into one.

The male gametophyte of *E. trifurca* shows a primitive condition in that it has retained two persistent prothallial cells. A comparison with the other groups of gymnosperms shows that in Cycadales the male gametophyte is less primitive in that it has retained only one prothallial cell, with perhaps occasionally another very evanescent one. Ginkgoales have one persistent and one evanescent prothallial cell. In Coniferales the Taxaceae and the Pinaceae show all grades in reduction of prothallial cells; *Podocarpus Hallii*, now being studied in this laboratory by Mr. L. L. BURLINGAME, frequently has as many as eight prothallial cells; Taxus and Torreya have none; Araucaria perhaps shows several prothallial cells; Pinus has two evanescent prothallial cells; and the Cupressineae none at all. According to PEARSON,[8] Tumboa has one prothallial cell. The testimony as to the Gnetums is doubtful; apparently some of them have one ephemeral prothallial cell and others none at all. *E. trifurca*, therefore, may be said to have retained a primitive character of its male gametophyte longer than Cycadales and Ginkgo, and longer than most Coniferales. It is possible that the number of prothallial cells will be found to vary in other species of Ephedra;

[7] COULTER, JOHN M. and LAND, W. J. G., Gametophytes and embryo of *Torreya taxifolia*. BOT. GAZETTE **39**: 161–178. *pls. A, 1–3*. 1905.

[8] PEARSON, H. H. W., Some observations on *Welwitschia mirabilis* Hooker *f.* Phil. Trans. Roy. Soc. London B **188**: 265–304. *pls. 18–22*. 1906.

STRASBURGER found one prothallial cell in *E. campylopoda* and JACCARD none at all in *E. helvetica*. The technique is difficult and it is not safe to assume the absence of prothallial cells until a very large number of thin sections have been studied. The Gnetales as a group appear to have retained the primitive character of the male gametophyte along with a very high degree of specialization of the female gametophyte. In general a form showing a number of highly specialized characters may be expected to have primitive characters still lingering somewhere, perhaps because of the very high specialization of the majority of structures.

The usual number of sperms or male nuclei in gymnosperms is two. That this reduced number is derived from a gymnosperm condition in which more than two sperms or male nuclei were produced in a single pollen tube is very probable. The comparative study of antheridia and archegonia of bryophytes and pteridophytes shows that the antheridium is less conservative than the archegonium. Possibly the retention of the usual two sperms or male cells in gymnosperms is dependent upon the conditions under which they function. In gymnosperms the female gametophyte appears to have two tendencies; first to collect the archegonia into groups—archegonium complexes—in a common archegonial chamber; second, to reduce gradually the number of archegonia, maturing the few which are left earlier and earlier in the history of the female gametophyte, until they mature immediately after the free nuclear stage; finally, the free nuclei become eggs. The first-named situation, which perhaps after all may be only one of the stages in the last-named series, is best shown by the Cupressineae; the second tendency is illustrated by the taxad-Gnetales-angiosperm series. In Cycadales the sperms in each pollen tube, with one exception as yet, are two and equal, both being discharged into an archegonial chamber in which the archegonia are grouped; and each sperm has an equal chance to function. In Cycadales the archegonia are on the whole numerous, and that the archegonial group has resulted from the coming-together of scattered archegonia and that the tendency is further to eliminate archegonia is shown by the recent studies of *Dioon edule* by CHAMBERLAIN,[9] who finds from

[9] CHAMBERLAIN, C. J., The ovule and female gametophyte of Dioon. BOT. GAZETTE 42:321–358. 1906.

five to one archegonia in a chamber. An exceptional case was noted in which there were two archegonial chambers, each containing five archegonia; also instances were noted in which no archegonia were produced.

In Ginkgoales the two equal sperms are discharged into an archegonial chamber usually containing two archegonia, although occasionally three may be present; one functional and one abortive archegonium are not infrequent. Here, as in cycads, conditions are favorable for the retention of equality of sperms, at least for a very long time.

In Coniferales the situation is variable. In *Podocarpus coriacea*, COKER[10] found unequal male nuclei. The long-necked archegonia vary from six to ten and are separated from each other by one or more layers of cells; and the jacket cells do not go to pieces at the time of fertilization. Since a pollen tube discharges its contents into a single archegonium, only one male nucleus can possibly function. In Taxus[11] and in Torreya[12] the male nuclei are unequal, and the latter perhaps throws some light on the Ephedra situation. In Torreya the usually solitary archegonium appears as soon as walls are formed; even for a time after fertilization the walls are extremely tenuous, but show no tendency to break down. The gametophyte situation therefore closely resembles that in Ephedra.

In Pinus the numerous archegonia are scattered as in Podocarpus, but differ in that each archegonium has an individual archegonial chamber. The second male nucleus has no chance to function unless it may occasionally fuse with the ventral nucleus. The male nuclei are reported by COULTER[13] to be equal in *P. Banksiana;* CHAMBERLAIN[14] reports them equal in *P. Laricio;* while in *P. Strobus* Miss FERGUSON[15] reports them as unequal. An examination of Miss

[10] COKER, W. C., Notes on the gametophytes and embryo of Podocarpus. BOT. GAZETTE 33:89–107. *pls. 5–7.* 1902.

[11] BELAJEFF, W., Zur Lehre von den Pollenschläuche der Gymnospermen. Ber. Deutsch. Bot. Gesells. 11:196–201. *pl. 12.* 1893.

[12] COULTER, JOHN M., and LAND, W. J. G., Gametophytes and embryo of *Torreya taxifolia.* BOT. GAZETTE 39:161–178. *pls. A, 1–3.* 1905.

[13] COULTER, JOHN M., Notes on fertilization and embryogeny of conifers. BOT. GAZETTE 23:40–43. *pl. 6.* 1897.

[14] CHAMBERLAIN, C. J., Oogenesis in *Pinus Laricio.* BOT. GAZETTE 27:268–280. *pls. 4–6.* 1898.

[15] FERGUSON, MARGARET C., Contribution to the knowledge of the life-history of Pinus. Proc. Wash. Acad. Sci. 6:1–202. *pls. 1–24.* 1903.

FERGUSON's drawings, which show some of the male nuclei very irregular in outline, suggests the desirability that conclusions as to the equality of irregular nuclei should be based on volume rather than on optical sections. Perhaps the male nuclei of *P. Banksiana* and *P. Laricio* are more resistant to the conditions which cause elimination of supernumerary cells than is *P. Strobus*.

In Cupressineae the tendency to mass the archegonia in a common group finds its highest expression; in Thuja the further tendency to eliminate some of the archegonia is also apparent. In this group, with one notable exception, the male nuclei are two, and in all, so far as yet seen, are equal in volume and have equal chances to function. There are good reasons for believing that if few male nuclei were discharged into the complex the number of archegonia would be reduced gradually because of failure to function. In most of the Cupressineae the relatively high number of archegonia in a complex seems to be retained by having several pollen tubes reach the complex.

A member of the Cupressineae gave us the first proof of what seems to have been the primitive condition of the gymnosperm pollen tube. In 1904 JUEL,[16] studying *Cupressus Goveniana*, found sometimes four, more frequently eight to ten, and occasionally as many as twenty male cells in each pollen tube. JUEL considers the female gametophyte an abnormal one, for in one instance only did it get beyond the free nuclear stage and produce archegonia. The conditions which enable these numerous male nuclei to function are certainly ideal. In order to retain the large number of male cells which JUEL rightly considers a primitive gymnosperm condition, it seems that for some reason the female gametophyte of *Cupressus Goveniana* early lost its conservatism and went a step beyond the archegonium complex and formed free eggs. It certainly shows an interesting transition from archegonia to free eggs, and illustrates the tendency among gymnosperms to eliminate archegonia. The massing of archegonia and final elimination of walls must have taken place in *Cupressus Goveniana* while the male cells were still numerous, for in no other way does it seem possible to preserve the large number of male cells. That JUEL did not observe these free nuclei functioning in no way invalidates this conclusion.

[16] JUEL, H. O., Ueber den Pollenschlauch von Cupressus. Flora **93**:56–62. *pl. 3.* 1904.

In this connection it may be well to call attention to *Microcycas calocoma*, recently studied by CALDWELL,[17] which shares with *Cupressus Goveniana* the distinction of having retained a large number of sperms, sixteen being discharged from a single pollen tube. Large numbers of clustered archegonia are scattered all over the female gametophyte; also in numerous cases multinucleate archegonia were noted. This last condition seems to show that Microcycas while retaining archegonia has also only partially established septation among the free nuclei. The writer believes that the multinucleate archegonia of Microcycas are homologous with the "archegonial tubes" of Tumboa, and that the female gametophyte instead of being, as CALDWELL believes, the most primitive in cycads, has attained almost if not quite to the level of Tumboa. The same tendency to eliminate archegonia that is shown in all other groups of gymnosperms holds for cycads in a marked degree.

The Gnetales present practically an unbroken series illustrating the disappearance of archegonia and the coming-in of the embryo sac. In *E. trifurca* the long-necked, slightly scattered archegonia are very similar to those of Podocarpus. The antipodal end of the gametophyte is strikingly like the same region of Torreya, but the micropylar end shows an advance over Torreya in having the cells more loosely arranged and the jacket cells sometimes simulating eggs. The persistence of two equal male nuclei is perhaps due to the fact that the nuclei of the jacket cells in most cases become free. Another taxad character of Ephedra is the absence of resin ducts. It must not be assumed because of these resemblances to taxads that Ephedra, and of course the other Gnetales, are an offshoot from the Taxaceae.

A comparison of the female gametophyte of Tumboa shows a close resemblance to that of Ephedra in the antipodal region, but the micropylar end shows a very decided advance. PEARSON (*l.c.*) finds that the "corpuscula" described by STRASBURGER and by him considered as archegonium initials are in reality multinucleate. He calls them "prothallial tubes" and considers the micropylar region as even more highly specialized than in Gnetum. The evidence obtained from a study of the tendencies of the female gametophyte of all groups of

[17] CALDWELL, O. W., *Microcycas calocoma*. BOT. GAZETTE 44:118–141. *pls. 10–13*. 1907.

gymnosperms leads the writer to conclude that the prothallial tubes of Tumboa are best regarded as partial septations among the free nuclei of the micropylar end, and that the condition shown by Tumboa is a necessary stage in the elimination of the archegonium and the evolution of the embryo sac. Such an explanation is in keeping with the tendencies of every gymnosperm group. Of the male nuclei of Tumboa we know nothing.

In *Gnetum Gnemon*, according to LOTSY,[18] the female gametophyte has the antipodal region compact, with all traces of walls lost in the micropylar end, and the free nuclei all functional eggs. This is an advance over the partially septate condition of Tumboa. Two male nuclei are produced, but LOTSY is silent concerning their equality; from the condition under which they function there can be little doubt that they are equal. In the embryo sac of *G. Rumphianum* LOTSY found slight traces of septation. In the species of Gnetum investigated by KARSTEN[19] all trace of septation of the embryo sac is absent at the time of fertilization.

If we arrange a representative series of gymnosperms based on the female gametophyte alone, the series will be as follows: Podocarpus with its long-necked scattered archegonia; Torreya with usually a single archegonium appearing almost immediately after the free nuclear stage and having the antipodal and micropylar regions only slightly differentiated; *Ephedra trifurca* with from one to three long-necked archegonia with jacket cells sometimes simulating eggs, with antipodal and micropylar regions sharply differentiated, the former compact, the latter feebly walled; Microcycas with a loosely organized gametophyte at time of fertilization and having archegonia in clusters and also clusters of multinucleate archegonia which in the opinion of the writer, based on a comparative study of gymnosperms, are partial septations among free nuclei; Tumboa with free nuclei functioning as eggs in the partially septate micropylar end of the gametophyte; *Gnetum Gnemon* with the antipodal region compact and the micropy-

[18] LOTSY, J. P., Contribution to the life-history of the genus Gnetum. Ann. Jard. Bot. Buitenzorg II. **1**:46–114. *pls. 2–11.* 1899.

[19] KARSTEN, H., Beiträge zur Entwickelungsgeschichte einiger Gnetum-Arten. Bot. Zeit. **50**:205–215, 221–231, 237–246. *pls. 5, 6.* 1892; also Zur Entwickelungsgeschichte der Gattung Gnetum. Cohn's Beitr. Biol. Pfl. **6**:337–382. *pls. 8–11* 1893.

lar region unseptate and containing free eggs; *G. Ula*, *G. ovalifolium*, and *G. verrucosum* with an embryo sac containing free eggs. *Cupressus Goveniana* is probably near the Tumboa level.

It seems that during the time the female gametophyte is passing from the scattered archegonium condition either to the archegonium complex or to the free-egg condition there will necessarily be a reduction in the number of male nuclei, because of the impossibility of more than one functioning as we now understand the meaning of the term function. When either the complex or the partially free-egg stage is reached, there can be no further reduction in the number of male cells remaining. From this it follows that the attainment of an advanced stage by one structure may result in the preservation of primitive conditions in another structure intimately connected with the highly specialized one. Research in forms showing some highly specialized structures is likely to uncover primitive conditions, which we are now too prone to dismiss lightly as "interesting abnormalities," but which are in reality stages marking progress.

The division of the fertilized egg to produce eight proembryonal nuclei is a common habit of gymnosperms. The failure of three to six of these free cells in *E. trijurca* is suggestive of the next stage, the direct production of a single embryo from the fertilized egg, which is characteristic of Tumboa, Gnetum, and angiosperms. That the old habit of producing several embryos is occasionally retained by Gnetum is shown by an observation by LOTSY, who says that occasionally, as the suspensor is pushing downward, branching of the embryo may occur.

The sporophyte characters of the Gnetales are so well known that it is needless to discuss them.

A comparison of all the characters of Gnetales seems to show that we have a fairly consistent group, the most primitive being Ephedra, the most advanced Gnetum, with Tumboa between, but perhaps nearer to Gnetum than to Ephedra. That the three are phylogenetically related seems certain.

There is no proof that the Gnetales have been derived from or are directly related to any living group. They have many characters of Coniferales, and if related to Coniferales at all it must be to the Taxaceae with which they have many things in common. Gnetales

must be regarded as a modern group and the possibility of the derivation of angiosperms from them is of great theoretical interest.

## SUMMARY

*Ephedra trifurca* is monosporangiate, but occasionally bisporangiate strobili occur.

The beginning of the staminate and ovulate strobili can be seen in December; the pollen is shed about April 1, and the ovulate strobili early in May.

The anthers, developing in acropetal succession on a compound strobilus, are surrounded by a perianth.

Two integuments, free from each other except at the base of the ovule, are present; the outer has four rudimentary bundles, the inner two.

The nucellus is free from the inner integument for more than half its length.

A conspicuous pollen chamber reaches down and lays bare the tip of the gametophyte, on which many of the pollen grains come to rest.

The pollen chamber is finally closed by a plug derived from the outermost layer of the gametophyte cells forming the bottom of the chamber; this plug prevents the escape of the embryo into the pollen chamber.

The pollen grain sheds its exine on contact with the mucilage in the pollen chamber or in culture media, and the body cell divides before the pollen tube appears.

Two male nuclei equal in volume and optical appearance are produced.

The female gametophyte is composed of two definite physiological regions, a loose micropylar and a compact antipodal one with haustorial cells at the base. A column of thin-walled cells containing much food extends from the base of the archegonia through the center of the gametophyte nearly to the haustorial region, marking out the line the embryo will follow.

Fertilization is possible within ten hours after pollination.

The walls of the jacket cells break down about the time of fertilization and their contents mingle with the cytoplasm of the egg; occasionally some of the jacket cells closely resemble the egg.

The second male nucleus disappears in the upper end of the egg and, as it disintegrates, minute cells appear, which are believed to be the joint product of the chromatin of the second male nucleus and the chromatin of some of the jacket cells; these minute cells at least foreshadow the endosperm of angiosperms, and may be called physiological endosperm.

The fusion nucleus gives rise to eight free nuclei, two to five of which produce suspensors.

Each functioning proembryo produces an embryo with a single suspensor.

Only one embryo reaches maturity.

If moisture is supplied the seed does not rest.

Six months elapse from the setting of the strobili to the "germination" of the seed.

Ephedra must be considered as the most primitive of the Gnetales.

NOTE.—Since this paper was in type, a paper by Miss BERRIDGE and Miss SANDY[20] on *E. distachya* has appeared.

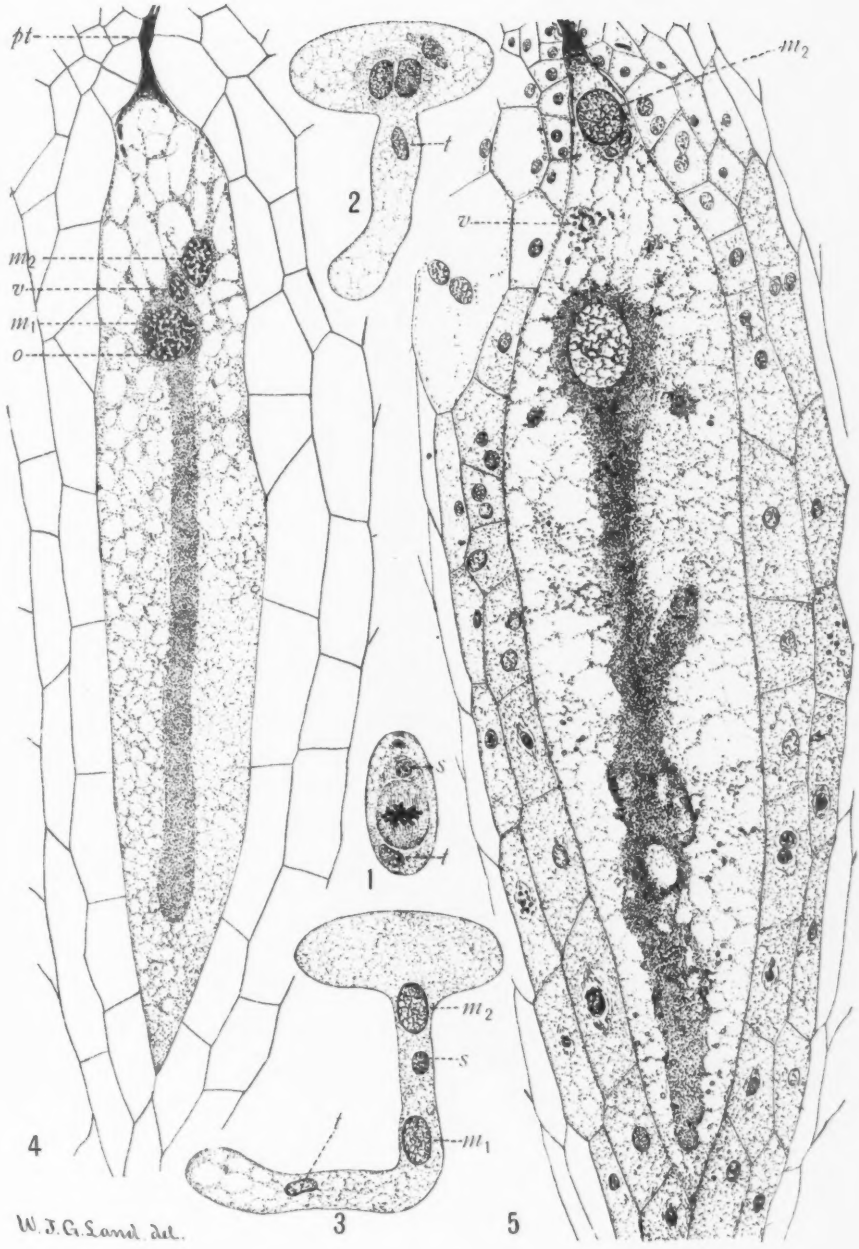
They report the male nuclei as being inclosed in a common cytoplasmic sheath and markedly unequal. The functional nucleus slips from this sheath, but was not observed to function. Two kinds of embryos—normal and abnormal—are described; the former being essentially as described for *E. trifurca;* the latter, if the interpretation of the authors is accepted, is unique among gymnosperms. The nuclei of the jacket cells divide amitotically, escape from the cells, fuse in pairs, and produce proembryos. "In some cases the embryos are merely enlarged jacket cells which project into the archegonium." The writer has repeatedly observed in *E. trifurca* appearances which at the first glance might be interpreted as they are by these authors; but on careful examination and comparison in several thousand ovules they were found to be proembryos formed in the usual way. The ovule of Ephedra is so delicate that a slight pressure before fixation is almost certain to result disastrously to the structures.

THE UNIVERSITY OF CHICAGO

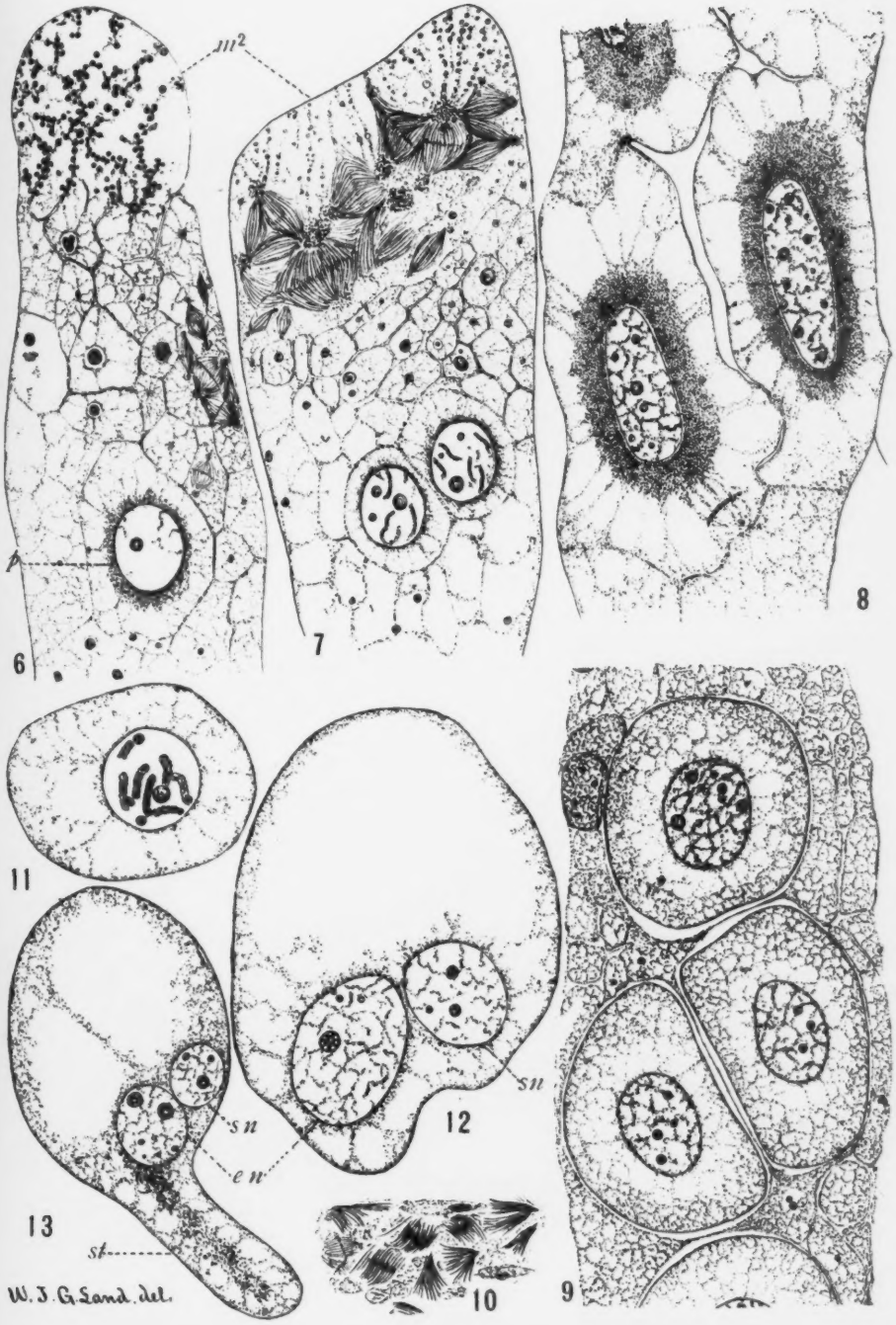
## EXPLANATION OF PLATES XX–XXII

All figures were drawn with the aid of an Abbé camera lucida and reduced one-half in reproduction. Abbreviations are: $m_1$, male nucleus which fuses with the egg nucleus; $m_2$, male nucleus which does not fuse with the egg nucleus; *s*,

[20] BERRIDGE, ETHEL M., and SANDAY, ELIZABETH, Oogenesis and Embryogeny in *Ephedra distachya*. New Phytologist **6**: 128–134, 167–174. *pls. 2, 3.* 1907.




LAND on EPHEDRA

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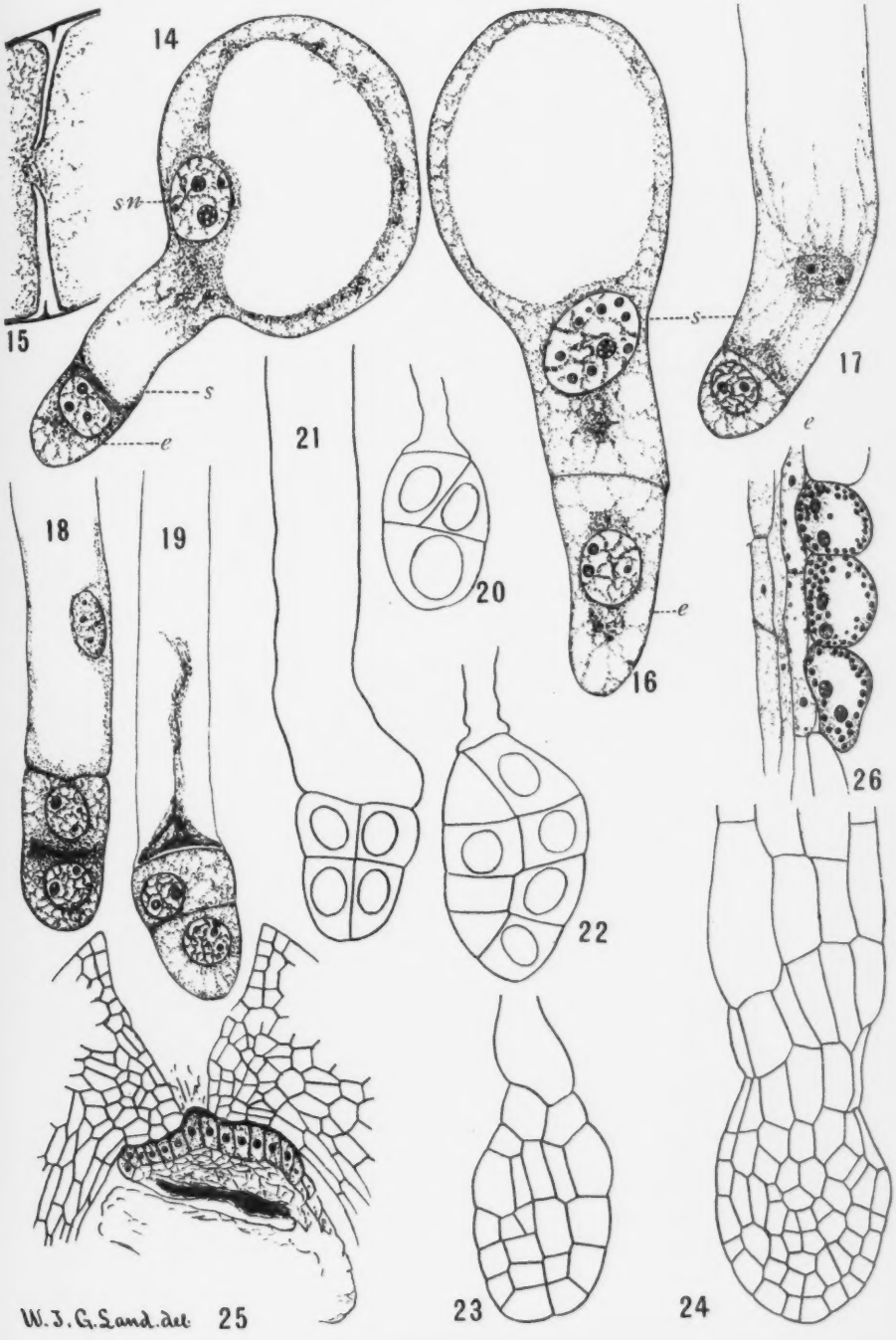



LAND on EPHEDRA

stalk-cell nucleus; *t*, tube nucleus; *pt*, pollen tube; *o*, egg nucleus; *v*, ventral nucleus; *p*, proembryonal nucleus; *st*, suspensor tube; *sn*, suspensor nucleus; *en*, embryo-initial nucleus; *s*, suspensor; *e*, embryo-initial cell.

*PLATE XX*

FIG. 1. Division of the body cell. ×500.

FIG. 2. Pollen tube with tube nucleus entering the tube; in the pollen grain are the two male nuclei lying side by side, above and to the right is the stalk nucleus. ×500.

FIG. 3. Pollen tube which has attained the maximum length in saccharose solution. ×500.

FIG. 4. Median longitudinal section through an archegonium showing end of pollen tube, the fusion of a male nucleus with the egg nucleus, ventral nucleus, and the second male nucleus. ×250.

FIG. 5. Archegonium showing fusion nucleus, fragments of the ventral nucleus. the second male nucleus, and jacket cells, some of which are dividing amitotically. ×250.

*PLATE XXI*

FIG. 6. Upper end of egg, showing second male nucleus, small cells derived from various sources, and one of two functioning proembryos. ×500.

FIG. 7. Upper end of egg showing a stage of the second male nucleus slightly later than in *fig. 6*, the small ephemeral cells, and two of four functioning proembryos. ×500.

FIG. 8. Proembryos, showing incomplete cleavage of the cytoplasm. ×500.

FIG. 9. Three of five functioning proembryos; jacket nuclei in the egg cytoplasm; free masses of cytoplasm; also masses of cytoplasm remaining after the proembryonal cells have been carved out. ×500.

FIG. 10. Disintegration of jacket cells. ×500.

FIG. 11. One of the proembryos before formation of the suspensor tube; the nucleus will shortly divide. ×500.

FIG. 12. Proembryo with two free nuclei and two papillae, the larger of which is the beginning of the suspensor tube. ×500.

FIG. 13. Proembryo with suspensor tube; the larger nucleus is the embryo-initial nucleus; the smaller will be the suspensor nucleus. ×500.

*PLATE XXII*

FIG. 14. Cleavage plane cutting off the embryo-initial cell from the tip of the suspensor tube. ×500.

FIG. 15. Detail of the cleavage plane separating the embryo initial from the suspensor. ×1900.

FIG. 16. A stage slightly later than *fig. 14*. ×500.

FIG. 17. Elongated suspensor with embryo-initial cell shortly before dividing. ×500.

FIG. 18. Division of embryo initial cell. ×500.

FIG. 19. A stage slightly later than *fig. 18*. ×500.

FIG. 20. Division of basal cell of the embryo; length of suspensor 1.84$^{mm}$. ×500.

FIG. 21. Division of apical cell of embryo. ×500.

FIG. 22. An embyro slightly older than *fig. 21;* length of suspensor 2.8$^{mm}$. ×500.

FIG. 23. Organization of dermatogen and beginning of the secondary suspensor; length of suspensor 3.1$^{mm}$, the longest suspensor found in any preparation. ×250.

FIG. 24. Embryo and secondary suspensor. ×250.

FIG. 25. Early stage in formation of the pollen-chamber plug. ×250.

FIG. 26. Epidermal cells of nucellus containing starch. ×250.

# THE DEVELOPMENT OF THE SEED IN THE PONTEDERIACEAE

W. C. COKER

(WITH PLATE XXIII)

A chance section of the young seed of Pontederia made several years ago presented a peculiar appearance in the antipodal end of the endosperm that led me to make an investigation of seed development in this family. I have been able to study *Pontederia cordata* and *Heteranthera limosum* in some detail, and *Eichhornia crassipes* to a less extent. Collections of Pontederia have been made in South Carolina and New York for several years. All my Heteranthera and Eichhornia material was collected in Jamaica during August 1900.

Examination of the literature of this family shows but one title dealing with its embryology. In 1898 SMITH (**14**) published a rather full account of seed development in Eichhornia and Pontederia, and gave one figure of the embryo sac of Heteranthera. Oddly enough, he completely overlooked the interesting peculiarity that appears in the endosperm of all three of the above genera, but otherwise, where parallel, my work, with a few exceptions, agrees with his. As SMITH did not attempt to give the development of Heteranthera, I have made a careful study of its ovular growth as a comparison with his work on Eichhornia and Pontederia. In the last two genera I have confined myself to the origin and development of the endosperm, which has the same peculiarity in all three types. HOFMEISTER (**9**) devotes a few lines to the development of Pontederia, but does not mention two sorts of endosperm.

## HETERANTHERA

The megaspore mother cell of Heteranthera is separated from the epidermis by a single tapetal layer, as is commonly the case in monocotyledons (*fig. 1*), and it reaches a very conspicuous size before its first division (*fig. 2*). The spindle of this division lies near the distal end of the cell, and cuts off a small upper and a large lower cell (*fig. 3*). Each of these now divides (*fig. 4*), and the result is a large, lower,

viable megaspore, with three small potential ones above. The two upper spores may either lie side by side (*fig. 5*), or one over the other (*fig. 5a*), agreeing in this respect with Eichhornia (SMITH). The division line between the upper two cells is very obscure, and as the cells soon show a sign of disorganization the cell wall separating them soon becomes invisible. In Eichhornia, SMITH describes two methods of megaspore formation from the mother cell; one as I have just described for Heteranthera, and another in which "the elongation of the ovule is accompanied by an elongation of the primary sporogenous cell without division of the latter. . . . The subsequent divisions in the elongated sporogenous cell take place in quick succession." He further says, "From a study of a large number of cases, similar to figs. 14 and 15, I have been led to believe that the four mother cells when formed in this way are seldom, if ever, separated by walls." I have noticed nothing in Heteranthera to indicate that there are two methods of megaspore formation. Walls are always formed in both first and second divisions, and the divisions occur in all cases as I have described.

Almost as soon as formed, the large lower megaspore commences to elongate and encroach on the ones above it, and after destroying them it pushes on through the tapetum and reaches the inner edge of the epidermis (*fig. 6*). Division of the nucleus now takes place and the embryo sac is soon organized (*figs. 6* and *7*). The structure of the sac is perfectly normal at this time; the synergids are rather sharply pointed and are decidedly denser than the egg; the antipodals are quite distinct, though small. SMITH states that in both Pontederia and Eichhornia the antipodals are ephemeral, disappearing early, but I have found this not to be the case in either Heteranthera or Pontederia. The lower end of the sac where the antipodals rest develops scarcely at all in the subsequent growth of the ovule, and remains as a little projecting pocket from an apparent basal invagination (*fig. 9*). In *fig. 8* is represented a sac at about the time of fertilization. The definitive endosperm nucleus is very large and conspicuous, being in fact as large as the egg or synergids.

At the stage represented in *fig. 9* a cell wall is present, cutting off the lower end of the sac into a separate chamber. In this chamber are a number of endosperm cells in addition to the three antipodals

which still occupy their little pocket. The origin of this lower tissue has not been determined in all stages for Heteranthera, but from its development in Pontederia there is no doubt that it arises from the division of the lower of the two nuclei produced by the first division of the definitive endosperm nucleus. A later stage of this lower endosperm is shown in *fig. 10*. The section shows five cells below the diaphragm in addition to the antipodals. It will be seen that the cells below are larger than those above the partition. As this tissue has been studied most carefully in Pontederia, I will not go into detail here in regard to it, as it seems the same in both cases.

The structure of the nearly mature seed of Heteranthera is of sufficient interest to warrant some description. The axial embryo extends nearly the whole length of the seed, and is surrounded by a large-celled endosperm, which, except in its immediate neighborhood, is densely packed with starch. In *figs. 11* and *12* are shown longitudinal and cross-sections of the seed coats and superficial endosperm. The nucellus is entirely destroyed except for the scarcely recognizable micropylar cap, and the aleuron layer of the endosperm lies in immediate contact with a heavy cellulose membrane secreted by the inner integument. The inner integument (**II**) is considerably crushed and its cells are so densely filled with deeply staining material that their outlines are not recognizable except in unusually favorable conditions. Beyond the inner integument is a still heavier cellulose membrane, represented by a white line in the figures. This membrane seems to be secreted by the cells of the outer integument, which remains permanently two layers of cells in thickness. The outer layer changes very little in appearance during the development of the seed, remaining thin walled and boxlike, with the long axis running parallel to the longitudinal axis of the seed. The cells of the inner layer, on the contrary, undergo great changes. They become much elongated, perpendicular to the long axis of the seed, and flattened in the contrary direction. The partitions parallel to the long axis of the cells are much higher at their ends than in the center, and their outer edges dip inward in a regular curve (*fig. 12*). As the cells of the outer layer follow this curve, the result is that the surface of the seed becomes channelled and ridged in a longitudinal direction. A longitudinal section of the seed taken near the top of a ridge would

appear as in *fig. 11*. Taken in a channel it would be the same except for the height of the inner layer of the outer integument. At the time represented in the figures, which is shortly before the full maturity of the seed, these large cells are found to be multinucleate, with nuclei and protoplasm in apparently normal activity. In *fig. 12* five nuclei are shown in a single section; they seem to have been formed by ordinary mitotic division. Note that, contrary to what might be expected, they are on the outer side, where the cell walls are thin.

### PONTEDERIA

As SMITH has followed the early development of the embryo sac in Pontederia, I have taken up its history at a point just preceding the formation of the endosperm. In *fig. 13* is shown a sac just before the division of the definitive endosperm nucleus. This nucleus lies very low, just above the antipodal pocket, and in this position it remains until its division. As a result of the first division, a cross-wall appears, cutting the sac into a large upper chamber and small lower one (*fig. 14*). The endosperm nuclei in each chamber now divide repeatedly and the protoplasm increases rapidly in amount. As already mentioned, the lower tip of the sac does not increase in size, and as the part above grows outward, and develops downward at the edges, the antipodal pocket comes finally to hang from the center of a considerable depression (*fig. 17*). The antipodals never divide, but they are not ephemeral, as described by SMITH. They are easily visible after cell walls have appeared in the endosperm.

In the upper chamber of the sac the development of the endosperm presents no great peculiarities; cell formation begins only after the sac has increased in size many times and the embryo has become conspicuous. In the lower chamber the protoplasm increases rapidly in extent and density (*fig. 17*), and the nuclei multiply by mitotic division until their number becomes quite large, fifteen appearing in the single section shown in *fig. 17*. Soon after cell walls begin to appear in the upper chamber, they are also formed in the lower, but in a much more irregular way. From the first the cells cut out of the basal endosperm are often multinucleate, while those above are uninucleate, as usual, when first formed. Another point of difference is that the cell walls in the antipodal endosperm soon become

much thicker than in the tissue above (*fig. 18*). As the growth proceeds their nuclei become very irregular, stain deeply, and show a tendency to fuse together. The protoplasm also becomes much denser than that of the upper endosperm (*fig. 18*). In *fig. 19* are represented several cells of the antipodal endosperm more highly magnified; they are from a stage slightly later than represented in *fig. 18*.

As the maturity of the seed approaches, and the upper endosperm begins to be filled with starch, the cell contents of the lower endosperm show signs of disintegration. The nuclei become deeply staining and irregular masses, and the protoplasm loses its distinctive structure and begins to disappear. No starch was found in these cells at any time, and at the maturity of the seed they appear, like the cells immediately above them, empty and somewhat crushed at the tip of the embryo.

The number of cells in the basal endosperm may become quite large. I have counted as many as forty-five in a single section, and there must have been in that case at least two hundred.

The seed coats of Pontederia are very much like those of Heteranthera, except that the outer integument is three cell-layers thick, with the cells of the two outer layers thin and boxlike, and those of the inner of the same height throughout, giving a smooth, instead of ridged, surface to the seed.

## EICHHORNIA

My material of Eichhornia was not sufficient to follow the development of the basal endosperm, but its presence is shown in my sections at several stages. It seems to be similar in every way to that of Pontederia and Heteranthera. The structure of the seed coats is exactly as in Heteranthera. The same figures would do for both.

## CONCLUSION

In looking over the literature of the subject I find but a single case where a tissue similar to this has been described, and there its origin has not been determined in detail. BILLINGS (1) has found that in *Tillandsia usneoides* there is formed in the lower part of the embryo sac, just above the antipodals, a tissue which in structure and position much resembles that of the Pontederiaceae. He does not

give the exact origin of this tissue, but says: "the nuclei resulting from the first divisions of the endosperm nucleus take position at either end of the sac, leaving, however, a few to form a thin parietal layer between. At the antipodal end, cell formation with walls begins at once, and a number of large cells form a tissue which stands out conspicuously in the cavity of the sac, which otherwise contains only a few free endosperm nuclei." Tillandsia is considered a near relative of the Pontederiaceae, and it does not seem improbable that the origin of this tissue is the same in both cases. A difference to be noted is that in Tillandsia the basal endosperm tissue, according to BILLINGS, is cellular from the first.

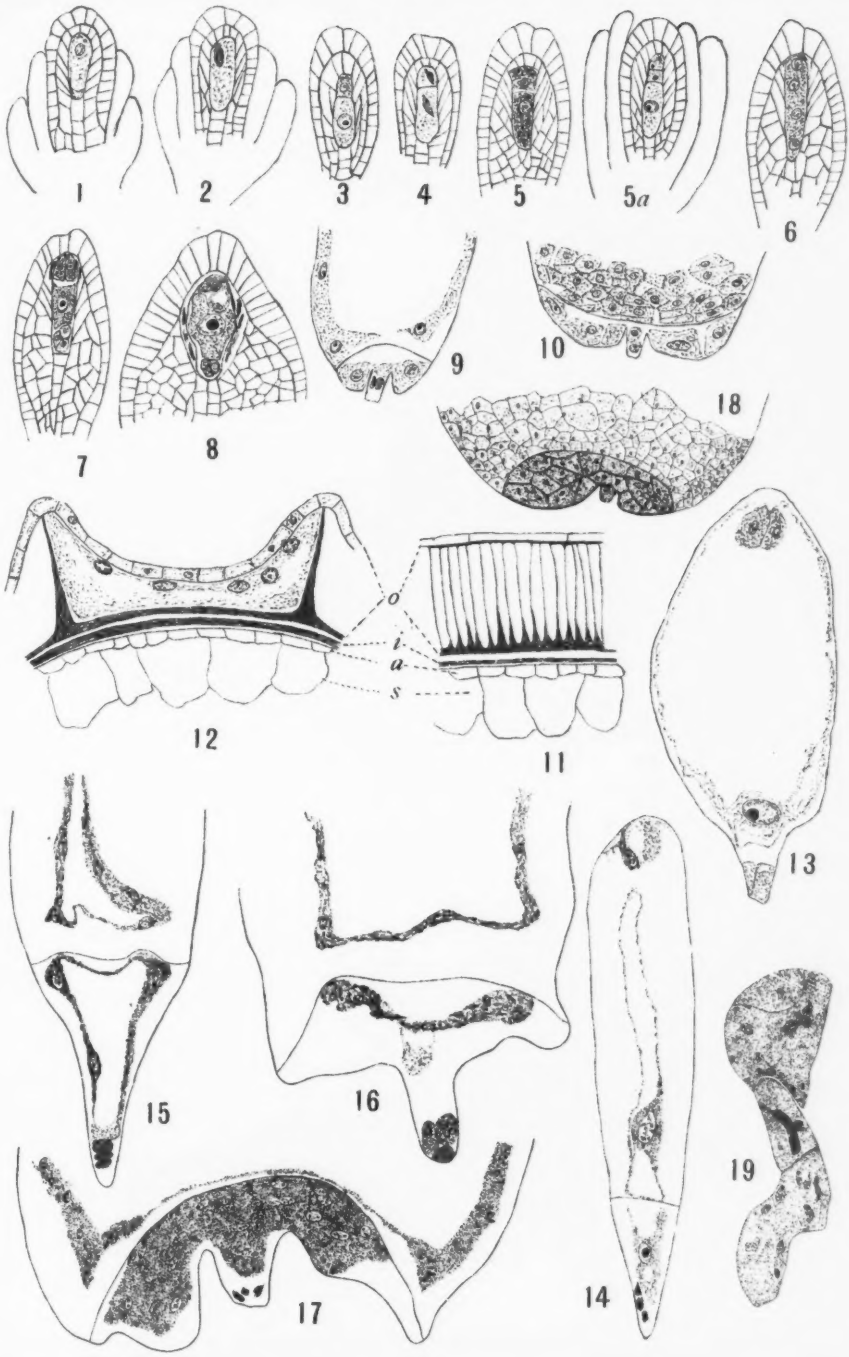
The appearance of the cross-walls at the lower end of the sac at the first division of the endosperm nucleus will at once recall a similar condition in Sagittaria (SCHAFFNER **13**), *Potamogeton pauciflorus* (WIEGAND **16**), and Ruppia (MURBECK **12**). In these cases, however, the nucleus in the lower chamber does not divide to form a tissue, as in the Pontederiaceae.[1]

In *Potamogeton foliosus*, according to WIEGAND (**17**), the young embryo sac has almost the same appearance as in Pontederia; but this structure is supposed to have come about in a different way. The wall that cuts off the lower end of the sac is derived from the division of one of the first four nuclei of the sac, its sister nucleus forming the three antipodals which occupy the little pocket below. The author thus regards the large nucleus which occupies the lower end of the sac as an antipodal, as it is the sister nucleus of the lower polar. No upper polar nucleus is supposed to be formed and the endosperm develops from the lower polar. This whole procedure is so peculiar that it seems open to some doubt until confirmed.

In *Potamogeton natans*, HOLFERTY (**10**) finds the embryo sac in general quite normal, but in two or three cases a large nucleus was discovered in the lower end of the sac, cut off above by a wall. He thinks it may have originated in the same way as I have described for Pontederia.

In *Castalia odorata*, *C. ampla*, *C. pubescens*, *Nymphaea advena*, *Brasenia purpurea*, and *Cabomba piauhiensis*, COOK (**5, 6**) has found that an antipodal haustorium, cut off from the rest of the sac by a

[1] In Sagittaria, according to SCHAFFNER, it may divide once or twice.




COKER on PONTEDERIACEAE

wall, contains a large nucleus which has the same origin as in Pontederia. This is also just what is found in the case of *Saururus cernuus* (JOHNSON **11**), but in none of these cases does the lower endosperm nucleus divide.[2]

In Limnocharis, according to HALL (**8**), the lower of the two nuclei formed by the division of the megaspore nucleus passes to the lower end of the sac, but does not divide to form antipodals and a lower polar nucleus. The upper polar nucleus, formed in the usual way, sinks to the lower end of the sac, and there divides without fusion (there being no lower polar). A cell wall resulting from this division cuts the sac in two. The nucleus left in the upper cell divides to form the endosperm, but the lower does not divide.

In both Naias and Zannichellia, CAMPBELL (**3**) has described a large nucleus near the antipodal end of the sac, which increases greatly in size, but does not divide. He did not determine the origin of this nucleus, but offers two suggestions, either that the large lower nucleus is the lower polar, or that it is the product of the first division of the definitive endosperm nucleus. The latter explanation seems most probable.

In *Elodea canadensis*, WYLIE (**15**) finds that there is generally present a large extra nucleus in the antipodal end of the sac. He says: "The sudden appearance of the extra nucleus in the antipodal group, when one has in mind the behavior of the polars, might suggest that these nuclei do not always fuse, and that one of them passes down to the lower end of the embryo sac and joins those in the antipodal pouch. The general evidence, however, is against this view. The irregular number of nuclei displayed here and the general arrangement of the cytoplasm about them would indicate that any increase in number has come from division among the antipodals, an activity in this region that often results from fertilization. In all embryo sacs studied at earlier stages the lower polar had passed out of the tip, and its return to the antipodal group seems improbable." Is it not more probable that this nucleus is formed as in Pontederia?

[2] COOK mentions a single division occurring once in *Nymphaea odorata*.

This discussion might be indefinitely extended by referring to the numerous cases of chambered sacs and other peculiarities of the endosperm of dicotyledons, but homologies are so improbable in such details that it is not necessary to cite them further. A good exposition of this subject may be found in COULTER and CHAMBERLAIN'S *Morphology of the angiosperms*.

An increase in the number of antipodals by division of the original three has been reported in a number of families, but, so far as I can discover, an endosperm tissue of the origin and character here described for the Pontederiaceae has not been before described. Its closest approach is probably to be found in Tillandsia.

CHAPEL HILL, N. C.

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## EXPLANATION OF PLATE XXIII

The magnifications given are for original drawings, which have been reduced one-half in reproduction.

*Figs. 1–12. Heteranthera limosum*

FIG. 1. Young ovule, showing megaspore mother cell. ×675.

FIG. 2. Mother cell in division. ×675.

FIG. 3. Result of first division of mother cell. ×675.

FIG. 4. The two daughter cells dividing again. ×675.

FIG. 5. Shows four megaspores; the two upper side by side. ×675.

FIG. 5a. Four megaspores in a row. ×675.

FIG. 6. Four-celled embryo sac. ×675.

FIG. 7. Eight-celled embryo sac. ×660.

FIG. 8. Embryo sac ready for fertilization. ×675.

FIG. 9. Lower half of older embryo sac showing antipodals and basal endosperm. ×450.

FIG. 10. The same after formation of cell walls. ×450.

FIG. 11. Longitudinal section of seed coats and superficial endosperm. *a*, aleuron layer; *i*, inner integument; *o*, outer integument; *s*, starchy endosperm. ×780.

FIG. 12. Cross-section of same. Letters as in *fig. 11*.

*Figs. 13–19. Pontederia cordata*

FIG. 13. Embryo sac just before division of definitive endosperm nucleus. ×670.

FIG. 14. Embryo sac just after said division. ×670.

FIG. 15. Later stage showing antipodals, basal endosperm, and upper endosperm. ×670.

FIG. 16. Still later stage of same. ×670.

FIG. 17. Just before beginning of cell-wall formation in endosperm. ×670.

FIG. 18. Showing both kinds of endosperm after formation of cell walls. ×150.

FIG. 19. Several cells of basal endosperm at about the same stage as *fig. 18*. ×670.

# BRIEFER ARTICLES

## SOME NEW WASHINGTON PLANTS

In his *Species and varieties*, DE VRIES makes the following statement: "[The subgenus Onagra] seems to be rich in elementary species, but an adequate study of them has as yet not been made. Unfortunately many of the better systematists are in the habit of throwing all these interesting forms together, and of omitting their descriptive study." A small collection of Oenotherae recently determined at the Gray Herbarium for W. N. SUKSDORF well exemplified the justice of this criticism, for although it lacked one of the two species of Onagra attributed to Washington in PIPER'S recent flora, it contained no less than three undoubtedly distinct species of this subgenus; one a very large-flowered plant of obvious relationship to *Oenothera Hookeri* T. & G., the second probably referable to *O. strigosa* (Rydb.) Mack. & Bush, the third a new species.

**Oenothera** (ONAGRA) **cheradophila** Bartlett, sp. nov.—Herba erecta ca. $1^{m}$ alta simplex vel in parte inferiore ramosa. Caulis vinaceo-purpureus deorsum albo-pilosus sursum subappresse strigosus. Folia caulina pallido-viridia lanceolata utrinque albo-strigosa; inferiora usque ad $3^{cm}$ lata $16^{cm}$ longa, apice acuta, basi in petiolum alatum brevem decurrentia, leviter remoteque denticulata, denticulis apice albis induratis; superiora parviora integerrima sessilia apice basique acuta. Bracteae foliis supremis similes sed eis parviores, maximae a capsulis maturis plerumque superatae infra mediam $6^{mm}$ latae $2.5^{cm}$ longae. Hypanthium $2.5^{cm}$ longum inferne ca. $2^{mm}$ crassum superne aliquanto crassius, extus patenter albo-pilosum. Calycis segmenta pallido-viridia densius subappresse albo-strigosa deltoidea $11–13^{mm}$ longa ad basin $4^{mm}$ lata, apicibus liberis, calyce nondum expanso, vix $1^{mm}$ longis acutiusculis. Petala flava saepe rosaceo-tincta $8^{mm}$ longa infra apicem $7.5^{mm}$ lata basi cuneata apice altiuscule retusa. Stamina corollam paulo superanta, filamentis planis ca. $6.5^{mm}$ longis sursum gradatim angustatis, antheris ca. $6.3^{mm}$ longis brevissime infra mediam affixis. Styli pars hypanthio exserta $2.5^{mm}$ longa. Stigma 4-partitum viride, ramis subteretibus carnosis ascendentibus $4^{mm}$ longis plerumque apices filamentorum vix attingentibus, raro paululo longioribus sed nunquam apices antherarum superantibus. Capsula matura prope basin $5–6^{mm}$ crassa $3^{cm}$ longa subquadrangula pallido- vel subolivaceo-viridis appresse albo-strigosa, ad apicem versus gradatim angustata et in rostrum $3^{mm}$ longum desinens; rostri segmenta, capsula dehisceta, recte divergentia. Semina brunneola prismatica $1.5–2^{mm}$ longa.

Type, *Suksdorf 5860* in Herb. Gray, low sandy river bank, Bingen, W. Klickitat County, Washington, 20 Aug. 1906. Other specimens which may be placed here are *J. S. Cotton 620*, sandy banks of river, Prosser, Yakima County, Washington, and (?) *L. N. Gooding 271*, river sand-bar, Middle Fork of Powder River, Johnson County, Wyoming. These were both distributed as *Onagra strigosa* Rydb., a clearly distinct species of which there is a cotype in the Gray Herbarium.

The strongest diagnostic features of the two plants are as follows:

| OENOTHERA STRIGOSA | OENOTHERA CHERADOPHILA |
|---|---|
| Bracts twice or thrice as long as the mature capsule, obtuse at base. | Bracts almost always considerably shorter than the mature capsule, acute at base. |
| Flower bud and hypanthium ochraceo-vinaceous, with sparse spreading pubescence. | Flower bud and hypanthium greenish yellow, pubescence more dense. |
| Free tips of calyx segments (in bud) $3^{mm}$ long. Petals $1.5–2^{cm}$ long. Color light yellow, fugaceous in wilting. | Free tips of calyx segments (in bud) less than $1^{mm}$ long. Petals $8^{mm}$ long or less. Color greenish yellow, intense, persistent after wilting. |
| Stamens shorter than the corolla. | Stamens usually slightly longer than the corolla. |
| Stigma a little exceeding the stamens, lobes flattish, spreading. | Stigma usually reaching only to end of filaments, teretish, ascending. |

In the case of both species the measurements were made from typical flowers of type material. At the end of the inflorescence, or on weak lateral branches, both *O. strigosa* and *O. cheradophila* often bear flowers which are greatly reduced in size and in which the petals are disproportionately small. In such flowers of the former species, the stamens are likely to be longer than the petals.

It is worthy of note that very little of the material which western collectors have distributed as *Onagra strigosa* Rydb. matches the plant from Pony, Montana, which Rydberg designated as the type of his species.

LEWISIA PYGMAEA (Gray) Rob., var. **aridorum** Bartlett, var. nov., caudice foliisque radicalibus eis formae typicae similibus; scapis brevioribus robustioribus a foliis longe superatis. Bracteae lanceolatae margine glandulis subatris longistipitatis praeditae. Pedicelli 1–3 quam bracteae paulo, nunquam duplo, longiores. Sepala usque ad $5^{mm}$ longa late ovata vel orbicularia margine denticulata, denticulis in glandulas longistipitatas atras desinentibus. Petala (ut in forma typica ex loco eodem) alba, anguste oblonga quam sepala dimidio longiora apice etiam glandulas stipitatas 3–4 gerenta. Capsula turbinata atro-brunnea $4.5^{mm}$ longa.

Type, *Suksdorf 5725* in Herb. Gray, dry, bare places in meadows, Mt. Paddo (Adams), Yakima County, Washington.

Unusually well marked as a variety by the extreme development of stipitate glands on the bracts, sepals, and petals. The variation is perhaps induced by habitat, for the typical form of the species (except for the fact that it has white petals) occurs at the same altitude on the mountain, growing intermingled with the variety to some extent, but more abundantly "in damp or wet sandy places." It may be that all of the Mt. Paddo material constitutes a species, of which the plant above described is a variety, differing from *Lewisia nevadensis* in its denticulate calyx segments and from *L. pygmaea* in its white corolla. Until a more significant difference from *L. pygmaea* comes to light, such a treatment is hardly to be justified, for in at least one species of the genus, *L. rediviva*, the color of the corolla is known to vary from deep rose to pure white.—H. H. BARTLETT, *Gray Herbarium*.

## SOME PERPLEXITIES OF THE INDEXER

During the last few years the work on the *Card index of new species, varieties, and forms of American plants* has necessitated the examination of a large number of taxonomic papers of both American and foreign authorship. Some of the perplexing questions which have arisen I wish to bring before the botanical systematist. It may be urged, as one correspondent has already suggested, that "the scientist writes for the student and not for the indexer." This is true, but is it the whole truth? In these days of multiplicity of publications it is well-nigh impossible for each student of systematic botany to review carefully all the literature which may possibly bear upon his subject or contain a new name in the group upon which he is working. Not long ago a publication relating to a state flora and containing more than 600 pages was carefully examined, with the result that six new combinations were found; but there was no indication whatever that these were new. Can each student spare time for this? With the aid of such works as the *Index Kewensis*, the *Index to North American fungi*, the *Card index*, etc., the student readily turns to the original descriptions of the plants in the group which interests him, and from articles containing these descriptions he finds references to the important works relating to his specialty. He is also able to ascertain whether a name or combination which he proposes to launch as new has been used previously. The relation of the index to the systematist is that of a catalogue to the worker in a library. Now the scientist is not the person who will take his time for index work, and even if he did he would be limited to the few groups

with which he is familiar, or should he undertake a larger field he would become, outside his specialty, only an indexer.

Indefiniteness and inaccuracy in publishing new names are the cause of much trouble to the indexer as well as the student, and result in many errors. One instance of this occurs where there is no indication to show that certain names in a given paper are new and many others cited in the same way have been published earlier. Even the student, in such cases, would be unable to discern the new from the old without the aid of indexes. Another perhaps just as misleading method of citation is to leave the "n. sp.," "in litt.," "in herb." or "MSS" attached to a name which has been already published. If the name appeared in the first edition of a work it is not a "n. sp." in the second edition; hence the inaccuracy. In 1894 Caloneis, a new genus of diatoms, was made. Under this new genus are 74 species, many of which are transferred from Navicula. Now, how are these species under a new genus given? One example, perhaps, will be sufficient: "*C. obtusa* W. Sm. (1853)." By reading the synonymy we learn that in 1853 W. Smith (who died in 1857) made the species *Navicula obtusa*, but he never heard of *Caloneis obtusa*, for the genus was not named until 1894, 37 years after his death. Is not then the citation "*C. obtusa* W. Sm. (1853)" misleading and therefore unscientific? Another form which gives rise to ambiguity is the unsupplemented use of the parenthetical authority. In many instances the author will cite the parenthetical author alone, leaving the author of the combination an unknown quantity. It may be a new combination, and if so the question arises whether a name can be considered published which is connected with no description and no synonym, but merely an authority given in parenthesis indicating that somewhere and some time during the last 150 years the author cited in parenthesis has used the specific name for this plant under another genus either for a species or variety. Too many examples might be given for this. In omitting the authorship of a combination some writers claim that the only one who should have the credit is the one who originally described the plant. The truly scientific worker does not think of credit, but he may well consider that the author of a correct combination is as important for reference to the future student as the author of the original name. Can it be that some authors omit their own names from new combinations because of modesty? In such a case why should the name be signed to the article in which they are published? When the authority of a combination is omitted there is no clue given to the place of the transfer, and one may search in vain without the aid of an index to find the date of the transfer or notes made in connection with it. Not infrequently the indexer must

spend hours searching, at best with doubtful result, for an earlier reference to, or a correct synonym for, a combination used with parenthetical authority only. If it is so important to give the authority for the original description of a plant, is it not just as important to give the name under which it was described, and the name of the one who has placed it in the correct genus?

In 1753 Linnaeus established the binomial system of nomenclature and this was followed until very recently. Now, however, a few of our botanists have reverted to the use of the trinomial, and not only for their own new names, but in citing authors of the past. Thus Dr. Torrey, Dr. Gray, and many others who never used this form are credited with it. Recently one of our younger botanists published the name *Spilanthes americana*, var. *parvula*, forma *parviflora*. Would it be a correct citation of this name to make it *Spilanthes americana parvula parviflora*, or fair to the author to attach his name to a combination so pre-Linnaean? One great objection to the trinomial is the indefiniteness of the third part; some use it as a subspecies, some as a variety, some as a form, some as a mutation, etc., and the reader or student is often left without any clue to what category it belongs. In a paper recently issued the preface states that the rank next below the species is a subspecies, thus making the third name of the trinomials used in this paper rank as subspecies. The author proceeds to make the third name of all his new trinomials subspecies and then cites all varietal names made by earlier writers as the third name of a trinomial. Does he thereby transfer varieties to subspecies and make new combinations which the student of the future and the indexer must consider? Certainly the names were not used in this form originally.

Just the opposite of the last point is the use of the binomial in the index to a periodical when the name on the page to which it is referred is not in accord: for example, *Prunella vulgaris scaberrima* occurring in an article is cited in the index to the volume as *Prunella scaberrima*. Is the latter, then, a new combination? If so, who is authority for it, for the name of the one who indexed the volume is never given?

The errors and inaccuracies of the past must remain. But the present and future systematic botanists can easily avoid them in their forthcoming works by (1) indicating clearly each new species, combination, or name; (2) not allowing a name to appear as new when it has been published previously; (3) always giving the correct authority, or, where the parenthesis is used, the correct double authority; (4) stating clearly the category to which each name, below the specific, belongs; and (5) in no way distorting or altering the rank of names attributed to other authors.—MARY A. DAY, *Gray Herbarium*.

# CURRENT LITERATURE

## BOOK REVIEWS

### Physiology and ecology

We take up Dr. CLEMENTS' new book[1] with favorable anticipations, ready to welcome a text of moderate size with the broad scope indicated by the title *Plant physiology and ecology*. The author tells us that the book is based upon his earlier *Research methods in ecology*, the manner of treatment being the same; i. e., first to consider the plant as an individual, establishing certain relations between the physical factors and its functions and form (physiology), and then to consider it as a member of a larger or smaller group of plants (ecology). "The proper task of physiology," says the author, "is the study of the external factors of the environment or habitat in which the plant lives, and of the activities and structures which these factors call forth. . . . . The sequence of study is consequently factor, function, form." So after selecting and describing some prominent factor—water, light, temperature, etc.—he proceeds to discuss its influence upon the various functions and organs.

Undoubtedly that method of treatment possesses marked advantages; but it may be seriously questioned whether our present knowledge is broad enough and exact enough to permit its successful use. And if it were, to write physiology in such a manner is peculiarly difficult. It requires thorough familiarity with the literature, especially that dealing with experimental morphology and irritability, clear conceptions of physical chemistry, and above all an instinctive avoidance of vague and inexact physiological cant. Certainly Dr. CLEMENTS' adoption of this method makes the physiological part of the book strikingly unconventional; the ecological part is less abnormal because ecology has proceeded from the first largely along such lines. And of the ecological part we have little to say, since it is essentially like that of the earlier book (reviewed in this journal, **40**:381. 1905).

Pedagogically the book leaves much to be desired. In the earlier sections the author wavers between textbook and manual; for beside the "experiments" in distinctive type (some of which are not experiments at all), the body of the text is partly subject-matter and partly directions to the instructor as to the management of his class, or to the student as to the materials and methods of his work.

In the very first chapter, on stimulus and response, the student is plunged

[1] CLEMENTS, F. E., Plant physiology and ecology. 8vo. pp. xv+315. *figs. 125*. New York: Henry Holt & Co. 1907.

into abstractions of which he can have no previous conception and regarding which he receives no adequate explanation. Thus, the tonic and stimulating action of external factors is discussed in a dozen lines on page 2 and nowhere else referred to. Distinctions the finest are drawn between normal and abnormal adjustment, adjustment and adaptation, growth and modification, that puzzle the reviewer; and it is hardly probable they would be more intelligible to the sophomore, for whom the book is intended.

Logically the arrangement of topics is sometimes peculiar. How is the student to comprehend the treatment of translocation and storage of food as an adjustment to light; and particularly in what sense can digestion, respiration, fermentation, the nutrition of non-green plants, growth, reproduction, pollen and pollination (treated at length), flowering, and fructification be looked upon as adjustments to temperature?

Dr. CLEMENTS is known as the proposer of many technical terms, of which few, fortunately, reappear in this book. But, what is even more confusing, he makes free to change the meaning of long-used terms. We had a pretty definite idea of what stimulus and response mean, but it does not fit when absorption, diffusion, transport, and transpiration are called responses to water stimuli. Imbibition is defined so as to include the entry of water into porous bodies. Cutinization is said not to coexist with epidermal hairs and is practically limited to xerophytes. The well-known actinometer masquerades as a photometer; heliotropic is distinguished from phototropic; chemosynthesis would never be recognized by the man who introduced the term; rootstalk, a word unknown to dictionaries, replaces the Anglo-Saxon rootstock. Aphototropic must surely be a typographic error, but nyctøtropic is doubtless an emendation. Reaction time and perception time have new and confusing definitions; but presentation time does not appear at all.

One serious danger inheres in Dr. CLEMENTS' method; there is a tendency to ascribe changes of function and form which have a very complex causation to a particular factor. One has only to examine the table of contents to find illustrations of this and the pages abound in examples. The offhand way in which some of the most elusive problems are settled by referring them to simple causes is appalling. The cause of cell division, the nature of polarity, the relation of turgor to growth, the mechanics of stomata are a few cases which the judge as it were dismisses with costs!

Many a pitfall, too, awaits the thoughtless in "explanations" which are merely a form of words that only cloak our ignorance. Absorption is "an inherent property of protoplasm;" "the need for the water of absorption increases toward the fibrovascular bundles," and "in similar fashion the demand for food materials increases toward the outer layers," so that "the general direction [of movement] is determined by these two *facts*" (sic?); the "stability of a species" prevents its modification in a new habitat—these are a few examples of meaningless phrases.

For the adopted mode of presentation an acquaintance with recent work is

a *sine qua non*. Yet some statements have a curiously antique flavor. PRINGSHEIM'S old screen theory of chlorophyll appears on one page and on the opposite one the modern theory is stated, but no decision as to their merits is reported. The amount of $CO_2$ in the air still appears as .04 per cent, and the starch formula as $C_6H_{10}O_5$. The work of two decades on solutions and osmosis is overlooked, for its bearing on the entrance of water, salts, and gases and on the movement of water and foods scarcely appears. The great advances in the last five years in the knowledge of perception, perceptive regions, and the interrelations of reactions find no reflection in this book, though they belong to its very essence.

Dr. CLEMENTS set himself a very difficult task; perhaps an impossible one, if we do not mistake the trend of recent study. That must be allowed for. Our main criticism, however, is not upon the choice of material for a brief treatise; it is against the attitude of mind that can tolerate vague explanations and invalid reasoning, and against a treatment of fundamental topics which is ineffective and not in accord with present knowledge.—C. R. B.

### Jost's Plant physiology

When we reviewed this book in its original German,[2] we expressed the opinion that it was "easily the best work on plant physiology in any language, for the general student." Further use of it has confirmed this opinion, though it has at the same time uncovered some weaknesses that did not appear on the surface. It still remains as adequate a presentation of the fundamental features of plant activity as is likely to be made by any one author. Though it lacks the suggestiveness and comprehensiveness of PFEFFER, and can by no means replace that indispensable work, its clear and picturesque style adapts it better to the advanced student and teacher. Our hope that it would be translated into English has just been fulfilled. The Clarendon Press secured the right of translation and Professor HARVEY GIBSON, of the University of Liverpool, is responsible for the work.[3]

There has been no editing of the author by the translator. Even the index is a translation. Dr. JOST himself has added some notes and embodied some corrections; but these are relatively few and brief, and might well have been a more marked feature of the English edition.

A literal translation was the aim, says the preface, but that certainly has not been attained. It is not difficult to find passages where wholly gratuitous changes have been made, qualifiers added or omitted, or variant expressions used, that leave the phrase far from a literal translation. For example: "hochstens ganz gelegentlich musste erwähnt werden . . . ." is rendered "we drew special attention to the fact . . . ." when the fact *was* only incidentally mentioned; and "wachstumsfördernde Wirkung" appears as "stimulating effects," with growth

[2] BOT. GAZETTE 37:390. 1904.

[3] JOST, LUDWIG, Lectures on plant physiology. Authorized English translation by R. J. HARVEY GIBSON. Imp. 8vo. pp. xiv + 564. *figs.* 172. Oxford: Clarendon Press. 1907. Cloth 21 *sh.*, morocco 24 *sh.*

not alluded to in the context. "Quite, or almost quite, impermeable" for "sehr schwer oder gar nicht permeabel" surely must be a typographic slip for "almost or quite impermeable." When JOST wrote "der im Wasser gelösten Moleküle und Ionen," why should it be translated, "of the molecules dissolved in water and of the ions," as though the ions were not dissolved in water? There are other slips of more serious nature. Thus, in describing the cohesion movements of the annulus of ferns and the anthers of seed-plants, to render "Füllwasser" by "imbibition water" makes the description inconsistent and unintelligible, for it is the water contained in the cell cavity, not the imbibed water of the walls, whose tension comes into play.

If these defects appear in a cursory examination one may suspect that a thorough comparison would reveal many others. Yet the total impression, without such a search, is quite the reverse, and it may be fairly said that on the whole the translation, though rather wordy, is good. The English may not be irreproachable, but it happily does not fall into the German idiom.

We note JOST'S lame excuse for retaining assimilation instead of adopting photosynthesis, pleading bad examples and the lack of a correlative term to apply to "nitrogen assimilation." A little boldness, pardonable in a leader, would have set aside the examples of PFEFFER and WIESNER, and a little ingenuity would have devised a new term, if amide synthesis and proteid synthesis were not acceptable. A bit more boldness in the translator would have at least harmonized the typography of citations with the better style of the original, and not overmuch would have modernized it completely. Now it halts between the traditional and the modern, losing much of the modest advance the author had made.

In makeup the book follows the general style of the Clarendon Press classics. The thicker but lighter paper, the solid type and slightly larger page, make a bulkier book, actually larger by some 60,000 words, fall within the compass of 554 pages of text as against 682 in the original. The rough-surfaced paper makes impossible the proper printing of the delicate cuts of the German edition, the halftones especially being mere smudges. The figures should have been remade for such paper. Yet we can almost forgive the blotchy cuts for the sake of the dull surface and the agreeable lightness of the book. But the price, as usual, goes away up—21 to 24 shillings as against about 15 marks—and imported copies at $7.75 will be almost too dear for general students. Yet it is well to have it available in English even at the exorbitant price.—C. R. B.

### The morphology of plants

DR. JOS. VELENOVSKÝ[4] has added to the lengthening list of general textbooks on plant morphology. Every such work represents some characteristic point of view, and that of VELENOVSKÝ seems to be that of the older morphology tinctured

[4] VELENOVSKÝ, JOS., Vergleichende Morphologie der Pflanzen. Vols. I and II. pp. 731. *pls.* 5. *figs.* 455. Prag: Fr. Řivnáč. 1905 and 1907.

by a modicum of the new. In fact, the author seems to regard modern morphologists as perhaps too narrow in their vision to possess the proper perspective for a general text. As a result, the two volumes now published are distinctly not up to date from the standpoint of modern morphology.

The first volume (pp. 277), issued in 1905, is introduced by general discussions of questions that are not current in modern laboratories, however interesting they may have been during the domination of the Goethean morphology. The main part of the volume consists of "Die Morphologie der Kryptogamen." The illustrations are well selected and many of them are original. The ground has evidently been well traversed in the laboratory but not in recent literature, for the text gives a distinct impression of long isolation. Why do so many botanists fail to see the incongruity of such a series of names for coordinate groups as "A. Thallophyten," "B. Charophyta," "Ç. Moose," "D. Gefässkryptogamen"?

The second volume (pp. 454), issued in 1907, presents "Die Morphologie der Phanerogamen," in so far as the vegetative organs are concerned. The main divisions are "Die Keimpflanze," "Die Wurzel," "Das Blatt," "Die Achse," and "Die Trichome." The general attitude is indicated by the subdivisions under "Seedlings," which are as follows: seedlings of polycotyls, of monocotyls, of stemless plants, of acotylous plants.

The third volume is to contain the morphology of the "phanerogam flower" and a system of classification, besides a general index.

The work will undoubtedly prove a most interesting one for reference, especially for the younger generation of morphologists.—J. M. C.

## MINOR NOTICES

**North American Flora.**—The first part of volume XXV has just appeared, being the fifth part published. It contains four of the sixteen families of Geraniales, the order with a synopsis of its families being presented by J. K. SMALL. The Geraniaceae, by L. T. HANKS and J. K. SMALL, contain four genera, a new genus *Robertiella* being founded on *Geranium Robertianum*. The species of Geranium number sixty-four, of which twenty-three are new, and Erodium and Pelargonium each contain six species. The Oxalidaceae, by J. K. SMALL, contain ten genera, *Hesperoxalis*, *Otoxalis*, and *Bolboxalis* being new and monotypic. The other genera are Oxalis (3 spp., 1 new), Ionoxalis (65 spp., 28 new), Monoxalis (2 spp., 1 new), Lotoxalis (11 spp., 1 new), Xanthoxalis (26 spp., 1 new), Biophytum (1 sp.), and Averrhoa (2 spp.). The Erythroxylaceae, by N. L. BRITTON, represented by the single genus Erythroxylon, contains twenty-eight species, one of which is new. The Linaceae, by J. K. SMALL, contain four genera, *Hesperolinon* being a new genus established on *Linum* § *Hesperolinon* Gray. The genera are Linum (5 spp.), Cathartolinum (48 spp., 10 new and all but one of the remainder transferred from Linum by SMALL), Hesperolinon (9 spp.), and Reinwardtia (1 sp.).—J. M. C.

**Physiological pedagogy.**—Though the notice is belated, a pamphlet by SCHLEICHERT,[5] illustrating the sort of instruction in plant behavior which he considers adapted to the eighth or ninth school year, deserves mention. The fundamental processes in germination, absorption, transpiration, photosynthesis, and respiration, are presented in simple fashion. There are appendices (1) giving a list of plants adapted for observation in the school garden; (2) on biological collections of plants; and (3) on simple methods (mostly devised by the author) for recognizing various plant stuffs.—C. R. B.

**Diatoms of the Pacific.**—MANN[6] has published the results of his investigation of the diatoms collected by the Albatross of the U. S. Bureau of Fisheries, during its fishery and deep-sea investigations in the Pacific in the years 1888–1904. The contribution contains a synoptical catalogue of the species, the data of the stations at which the diatoms were collected, and a very useful bibliography. The 51 genera recognized include 298 species, 41 of which are described as new. The large genera are Navicula (54), Coscinodiscus (34), and Biddulphia (30).—J. M. C.

**Portraits of botanists.**—The third and fourth parts of DÖRFLER'S series of portraits of botanists have been issued. A description of the general character of the series was given in this journal (**42**:496. 1906) upon the appearance of the first two parts. The third part contains the portraits of RUDBECK, LINNÉ (4), HALLER (2), SCOPOLI, JACQUIN, and ALLIONI; the fourth part, WULFEN, INGEN-HOUSZ, HEDWIG, GAERTNER, KÖLREUTER, SCHREBER, PALLAS, EHRHART, THUNBERG, and LAMARCK.—J. M. C.

**Engler's Syllabus.**—That the development of our knowledge of the relationships of plants is very rapid is evidenced by the appearance of the fifth edition of ENGLER'S *Syllabus*.[7] The most recent information about Cycadofilices was even so recent that it was inserted by means of a paster. It is a great boon to botanists that this publication keeps so well abreast of current knowledge, for it is a volume of daily consultation in every working laboratory.—J. M. C.

**Flora of the Canadian Rockies.**—Miss FARR[8] has brought together the plants of this interesting region collected by herself and reported by other botanists, and has presented a list of 763 species of vascular plants, 38 of which are pteridophytes, and 15 gymnosperms.—J. M. C.

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[5] SCHLEICHERT, F., Bieträge zur Methodik des botanischen Unterrichts (Sammlung Naturwissenschaftlich-pädagogischer Abhandlungen, Band **2**, Heft 3). Imp. 8vo. pp. iv+48. Leipzig and Berlin: B. G. Teubner. 1905. *M* 1.

[6] MANN, ALBERT, Report on the diatoms of the Albatross voyages in the Pacific Ocean, 1888–1904. Contrib. U. S. Nat. Herb. **10**:221–419. *pls. 44–54.* 1907.

[7] ENGLER, ADOLF, Syllabus der Pflanzenfamilien. pp. xxviii+248. Berlin: Gebrüder Borntraeger. 1907. *M* 4.40.

[8] FARR, EDITH M., Contributions to a catalogue of the flora of the Canadian Rocky Mountains and the Selkirk Range. Contrib. Bot. Lab. Univ. Penn. **3**:1–88. 1907.

## NOTES FOR STUDENTS

**Vegetation of the high Andes.**—WEBERBAUER draws this picture of the andine vegetation:[8a] In the high Andes of central Peru most plants are not structurally xerophilous, and dessication by the high altitude is much restricted by the abundant precipitation and the prevalence of clouds. Were the danger of drying so great, one would expect that the moistest locations would bear the most species and the most vigorous forms, and that on the moist ground the vegetation would ascend higher than elsewhere. But the vegetation of the high Andes presents no such picture; the high-andine moor contains fewer species than the meadows of cushion and rosette plants, and none of those stouter forms which in their aspect recall the lower regions—the shrubs, tufted grasses, and tall-stemmed herbs; these grow by preference rather on the dry slopes, the stony ground, and the rocks; and upon a stony substratum the limits of vegetation run higher than upon well-watered ground. The explanation of these phenomena is to be sought, in my opinion, in the temperature of the ground. By the low temperature of the soil many plants are kept away from the moors, while the simultaneous high temperature of the rocks is favorable for vegetation. That temperature of the soil at these elevations plays an important rôle is indicated by the horizontal position of roots, referred to in an earlier paper,[8b] and by the very aspect of the vegetation.—C. R. B.

**Transmission of tropistic stimulation.**—FITTING[8c] has undertaken to ascertain in what manner the tissues of perception and reaction are connected that the stimulus indirectly determines the direction of the curvature. The work consists mainly of a study of phototropic response (coleoptile of the grasses, chiefly Avena) as affected by more or less complete disconnection of the tissue between the apex and base. Wounding by transverse incision or by splitting does result in a feeble traumatic response, but neither the growth nor the capacity for perception and response is significantly affected. It may be said that the reliability of the author's conclusions hinges largely upon the truth of this statement, but the reviewer finds no reason for doubt and the work of ROTHERT is in accordance with it. The author shows that whatever course transmission is compelled to follow, the curvature is entirely independent of the unilateral occupation of the perceptive organ by the stimulus. Individual segments of halved or quartered coleoptiles will respond if the segment includes a small portion of the apex. Even in leaves from whose central portion a large square of tissue has been removed, transmission occurs. The stimulation seems to spread with equal ease across or along the organ. The author regards it highly probable that phototropic transmission

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[8a] WEBERBAUER, A., Weitere Mitteilungen über Vegetation und Klima der Hochanden Perus. Engler's Bot. Jahrb. **39**:449–461. *pls. 4, 5.* 1907.

[8b] *Op. cit.* **37**:60 ff. 1905.

[8c] FITTING, HANS, Die Leitung tropistischer Reize in parallelotropen Pflanzenteilen. Jarhb. Wiss. Bot. **44**:197–253. *fig. 26.* 1907.

proceeds through the living substance. The paper maintains the high standard we have come to expect from this author.—RAYMOND H. POND.

**Ants and plants.**—ULE has already smitten hard the theory of myrmecophily, according to which the complicated cavities and other structures have arisen by natural selection through the protection afforded by the symbiotic ants against injury by other ants and browsing animals. Now he concludes[9] that it must be given up almost entirely, because his more extended observations in the Amazon region, where such plants most do congregate, cannot be reconciled with the theory.

The ant plants grow most abundantly in flooded regions, where no leaf-cutting ants are found; the devastation by leaf-cutters is not so extensive as it has been reckoned by VON IHERING; the weapons of the protective ants are not efficient, especially against thick-skinned animals, and are not capable of such wounds as those of many other ants; and the protection afforded, while possibly advantageous in the struggle for existence, is not sufficient to call forth the elaborate cavities and other "adaptive" structures. In explanation of the symbiosis, ULE would rather lay stress upon the initiative of the ants, which chose aptly the plants that suited their needs, and perhaps have modified them by the intimate and constant symbiosis, in much the same way as man has improved the wild ancestors of his useful plants. But the cavities must have *originated* through deeper-lying causes.

ULE adds a list of the ant plants found on his Amazon expedition, which includes 46 species in 11 families, the Melastomaceae having 17, and the Leguminosae and Moraceae each 7. He figures the extraordinary "tubers" (really Dischidia-like pitcher leaves) of *Polypodium bifrons* Hook., which serve as water sacs and incidentally as dwellings for *Azteca Traili*.

A still more vigorous objection is made by VON IHERING,[10] who has studied the relations between *Cecropia adenopus* Mart. and its inhabitant, *Azteca Muelleri* Emery, in a much more thorough fashion than was done by FRITZ MUELLER and SCHIMPER. He has given special attention to the life-history of the ants and the metamorphosis of their nests. The "Müllerian bodies," which grow on the leaf bases, are not used to feed the larvae but as food by the ants themselves, though they are by no means restricted thereto. The small tumors, produced from the inner parenchyma near the entrance to the hollow of the stem as a result of gnawing the prostoma open, contain sugary and fatty substances and serve as food. VON IHERING rejects absolutely the elaborate conceptions of "mutualism" and "myrmecophily," and declares in so many words that *Cecropia adenopus* can get along quite as well without ants as a dog can without fleas. He considers the ants mere parasites, completely adapted to this plant, without which they cannot prosper at all; but all the plant "adaptations" are like those that hosts everywhere show to parasites.—C. R. B.

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[9] ULE, E., Ameisenpflanzen. Engler's Bot. Jahrb. 37:335–352. *pls. 5, 6.* 1906.

[10] IHERING, H. VON, Die Cecropien und ihre Schutzameisen. Engler's Bot. Jahrb. 39:666–714. *fig. 1. pls. 6–10.* 1907.

**Regeneration and polarity.**—In a paper which has escaped our attention until now (on account of the removal of Dr. McCALLUM to Arizona), VÖCHTING[11] takes up again these problems, stimulated by KLEBS's report[12] of the surprising ease with which he was able to reverse the polarity of certain willows—the unattained goal of many a previous experimenter. Much of this paper is devoted to a reexamination of KLEBS's work, which VÖCHTING is unable to confirm and against whose conclusions he enters vigorous protest. He finds no evidence that polarity has ever been reversed by any experimental condition; he still holds that it is a special property of the body, founded in the very structure of the egg, and that it is not induced (as PFEFFER thinks) by any external agent. This conclusion seems to be inevitable, when one observes the extremely different positions and conditions under which eggs develop into embryos. In regeneration the various natural or experimental conditions produce each an appropriate effect, according as they cooperate with or oppose or do not affect polarity. In a supplementary note, VÖCHTING expresses his complete agreement with the conclusions reached by McCALLUM.[13]

Miss KUPFER also attacks the problems of regeneration,[14] and after reporting in some detail her experimental work, concludes that polarity is less rigid than is generally believed, the "disposition to form roots being much more generalized . . . . and more easily energized than the power to form shoots;" and that regeneration is dependent upon an adequate supply of food.

Not satisfied because various theories of regeneration fail to account for the formation of one or another kind of organ "when each of the conditions previously ssigned as the causes of regeneration has been fulfilled," she proposes a modification of the *Stoff und Form* theory, postulating the existence of localized root-forming and shoot-forming enzymes. To this the same objections apply as to the older form of this theory; but the author has vague hopes that it may be capable of experimental test, though "no results ready for publication have as yet been obtained." One neglected assumption (also made by GOEBEL) needs quite as thorough investigation as any, namely, that food will accumulate at any point *before* growth begins.—C. R. B.

**Ultramicroscopic structure.**—GAIDUKOV continues his studies with the ultramicroscope, describing in a late paper[15] the appearances in starch grains, cell walls, and protoplasm.

After an introduction upon the principles involved in the various forms of the

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[11] VÖCHTING, H., Ueber Regeneration und Polarität bei höhern Pflanzen. Bot. Zeit. 64[1]:101–148. *pls. 5–7.* 1906.

[12] KLEBS, G. Willkürliche Entwicklungsänderungen bei Pflanzen 96 ff. Jena. 1903.

[13] McCALLUM, W. B., Regeneration in plants. BOT. GAZETTE 40:97 241. 1905.

[14] KUPFER, ELSIE, Studies in plant regeneration. Mem. Torr. Bot. Club 12:195–241. *figs. 13.* 1907.

[15] GAIDUKOV, N., Ultramikroskopische Untersuchungen der Stärkekörner, Zellmembranen, und Protoplasten. Ber. Deutsch. Bot. Gesells.

instrument, he reports that he could see in the starch grains concentric or excentric rows of particles, for which he uses NÄGELI's term micellae. These are separated by spaces which are optically empty or possibly contain amicroscopic matter. On swelling, the micellae separate more and more and lose their regular arrangement. Cell membranes show an essentially similar structure, cellulose walls showing very small pale particles, woody and corky walls, on the contrary, having brilliant and large micellae.

Living cytoplasm is a hydrosol complex. The micellae show active Brownian movements, oscillation, wiggling, collision, recession, etc., even when actively streaming. The better nourished the cytoplasm is, the more numerous and closer are the micellae and the less the amplitude of the movements; and *vice versa*. By a thin hydrogel layer, a coagulum formed by the electrolytes present in the water of the cell wall and vacuole, this hydrosol is protected against further injury by coagulation or against excessive solution by water. The healing of this hydrogel layer or ectoplast after taking in or extruding solid particles (as in Amoeba) is explained as due to coagulation of the micellae of the hydrosol at the spot exposed by the break to the action of electrolytes. At the death of the protoplast there arises a hydrogel complex. The nucleus consists of a hydrosol complex poor in water. The chromatophores resemble rather the hydrogel complex. Microsomes consist of several micellae and are comparable to the *Micellarverbänden* of NÄGELI.

So far as these preliminary accounts show, GAIDUKOV is not merely reporting what he sees, but is making many assumptions as to their interpretation.—C. R. B.

**Germination of fern and moss spores.**—LAAGE[16] has made a somewhat extended study of the germination of fern and moss spores in darkness. By using both organic and inorganic solutions as culture media he was able to germinate *Osmunda regalis* and thirteen out of sixteen species of Polypodiaceae in darkness. Germination and starch formation were obtained in Osmunda grown in distilled water without the influence of light. $Fe_2Cl_6$ and $FeSO_4$ stimulated germination and cell division in Osmunda in darkness. Of all the Polypodiaceae, sown in darkness, *Pteris aquilina* and *Scolopendrium officinarum* germinated best, and *Asplenium lucidulum* and *Polypodium aureum* not at all. Contrary to the results of HEALD, higher temperatures were found to be detrimental to germination in the absence of light. No chlorophyll was produced in darkness, although reported by SCHILLING. The osmotic effect of the solutions on germinating spores was marked. Abnormal forms of rhizoids developed in distilled water cultures, and in 4 per cent. Knop's solution they were suppressed, while the young prothallia were deformed. A number of instructive tables showing the effect of solutions of different strengths on germination and number of cells produced in darkness are given. Of the mosses, Funaria and Bryum were germinated in darkness by using very attenuated solutions of inorganic

[16] LAAGE, A., Bedingungen der Keimung von Farn- und Moossporen. Beih. Bot. Centralbl. **21**:77–115. 1907.

salts as culture media. *Polytrichum commune* could neither be germinated in darkness in the solutions of inorganic salts nor by the addition of organic iron salts to the fluids. Moss spores have been germinated in darkness under chemical stimuli by both HEALD and TRÉBOUX. LAAGE is the first to report germination of fern spores by means of chemical stimuli.—W. J. G. LAND.

**Artificial apospory in ferns.**—GOEBEL[17] has published the result of his studies on apospory in ferns and on regeneration in Vicia and Phaseolus. The main part of his paper is devoted to apospory and a very brief account is given of regeneration in Vicia.

Contrary to BOWER'S statement that attempts to induce apospory, though successful in certain mosses, have been entirely without results in ferns, GOEBEL succeeded in inducing artificially aposporous prothallia from primary leaves of sporophytes in many forms among ferns, such as *Aneimia Drageana*, *Alsophila van Geertii*, *Ceratopteris thalictroides*, *Gymnogramme chrysophylla*, *Polypodium aureum*, *Pteris longifolia*, and others. On certain parts of the under surface, of margin or of petiole of the primary leaf, there are produced prothallia, sporophytes, or even structures which really seem to be intermediate between sporophyte and prothallium by their having stomata and antheridia.

GOEBEL drew three conclusions from his studies: (1) regeneration is more active in a young leaf than an older one; (2) the sporophytic structure is not the constant product of regeneration; (3) there seems to exist no great difference between the nuclei of prothallia and those of sporophytes, and accordingly no sharp distinction between the $x$ and $2x$ generations. He adds, one might regard the prothallium, phylogenetically, as a rudimentary leaf bearing sexual organs, though to determine this question further investigation is necessary.

Among the three conclusions drawn by GOEBEL, the third is a great problem. He does not enter in his paper into nuclear detail at all; therefore, cytologically it is still an open question.—SHIGÉO YAMANOUCHI.

**Items of taxonomic interest.**—W. H. BLANCHARD (Torreya **7**:97–102. 1907), in discussing the eastern species of Amelanchier, describes 2 new species.—N. L. BRITTON (*idem* 102) has described a new Mexican species of Ribes.—H. D. HOUSE (*idem* 133–136), in a second paper on southern violets, has described a new species and a new hybrid.—W. H. BLANCHARD (*idem* 139, 140) has described a new Rubus (red raspberry) from Vermont.—E. BRAINERD (Rhodora **9**:93–98. 1907) has begun a presentation of the older types of North American violets.—L. M. UNDERWOOD (Bull. Torr. Bot. Club **34**:243–262. 1907), in continuation of his studies on "American ferns," has published a preliminary review of the N. Am. Gleicheniaceae, all the species being referred to Dicranopteris, in which 18 species are recognized, 5 being described as new, and all the rest being transferred.—C. L. SHEAR (Bull. Torr. Bot. Club **34**:305–317. 1907) has described 21 new

[17] GOEBEL, K., Experimentell-morphologische Mitteilungen: 1. Künstlich hervorgerufene Aposporie bei Farnen. 2. Ueber die Bedingungen der Wurzelregeneration bei einigen Pflanzen. Sitz. Kön. Bayer. Akad. Wiss. **37**:119–138. *figs. 13.* 1907.

species of fungi, most of which were discovered during his studies of the fungous diseases of the cranberry. Three new genera are described: *Plagiorhabdus* (2 spp.), *Bothrodiscus*, and *Acanthorhynchus*.—O. STAPF (Jour. Linn. Soc. Bot. **38**:6–17. 1907) has established a new genus (*Hallieracantha*) of Acanthaceae, to include species from Borneo and the Philippines heretofore referred to Ptyssiglottis; 19 species are recognized, 11 of which are new.—R. LAUTERBORN (Ber. Deutsch. Bot. Gesells. **25**:238–242. 1907) has described a new genus (*Thioploca*) of sulfur bacteria, belonging to the Beggiatoaceae.—J. M. C.

**The male gametophyte of the podocarps.**—JEFFREY and CHRYSLER[18] have been able to examine the male gametophyte of certain species of Podocarpus and Dacrydium, as well as of Agathis, obtained from New Zealand and Java, so far as material preserved in alcohol or formalin would permit. The conspicuous feature is the development of a prothallial tissue, by division of the two original prothallial cells, consisting in some cases of as many as eight cells. The walls of this tissue break down and the nuclei are freed, even from their cytoplasm, and swarm into the pollen tube. The authors do not regard this as a primitive feature, but consider the "ground plan" of this development as indicating the derivation of the podocarps and araucarians from an ancestral stock allied to the Abietineae. This feature also indicates that the podocarps and araucarians may be more nearly allied than has been supposed.—J. M. C.

**Infection experiments with mildew.**—REED[19] has been investigating the question of "physiological species" among the mildews. Recent work on mildews has indicated that each genus, and often each species of host plant, has its own particular specialized form. Infection experiments were conducted with twenty-three different varieties of commonly cultivated cucurbits, representing five species and three genera (Cucurbita, Cucumis, and Lagenaria). Each of these hosts was readily infected when inoculated with the conidia taken from any other. There was no difference in the infecting power of the mildew on the different species and genera, and there is no evidence of any specialization in the mildew of the Cucurbitaceae.—J. M. C.

**A lycopod with a seedlike structure.**—Miss BENSON[20] has investigated the reproductive structures of BERTRAND'S *Miadesmia membranacea*, a herbaceous paleozoic lycopod. The megasporangium develops a single thin-walled spore, "which in development and structure resembles an embryo sac and germinates *in situ*." The sporangium is surrounded by an integument with a small micropyle, which is surrounded by numerous long processes of the integument that "formed

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[18] JEFFREY, E. C., and CHRYSLER, M. A., The microgametophyte of the Podocarpineae. Amer. Nat. **41**:355–364. *figs.* 5. 1907.

[19] REED, GEORGE M., Infection experiments with the mildew on cucurbits, *Erysiphe cichoracearum* DC. Trans. Wis. Acad. Sci. **15**:527–547. 1907.

[20] BENSON, M., *Miadesmia membranacea* Bertrand; a new paleozoic lycopod with a seed-like structure. Abstract read before Roy. Soc. London, June 13, 1907.

a collecting and incubating apparatus for the microspores." The "carpellary leaf" was shed at maturity and resembles a winged seed.—J. M. C.

**The flowering plants of the Mesozoic.**—In a recent address SCOTT[21] has brought together the recent discoveries among the Cycadophytes, especially those of WIELAND among the Bennettitales, and has seen in the structure of their strobili strong suggestions of angiospermous flowers. Such a connection would suggest the possibility that the angiosperms may have been derived from the Filicineae by a "short cut;" that the evolution of the angiospermous flower was a process of reduction; and that the presumption that the simplest angiospermous flowers are the most primitive is no longer tenable.—J. M. C.

**The Taxoideae.**—Miss ROBERTSON[22] has brought together the results of the recent researches among the Taxineae, with the view of reaching some conclusion as to the vexed question of phylogeny. She reaches the conclusion that the group has retained many relatively primitive characters, but has become considerably specialized; that it may be regarded as an offshoot from the Cordaitales, which in turn are derived from the Cycadofilices. The author also suggests that the "female flower" of Taxus "more closely recalls that of Cordaites than that of any other known plant."—J. M. C.

**Embryology of Rhizophora.**—COOK[23] has succeeded in securing some material for the study of the embryology of *R. Mangle*. Only one of the four ovules reaches the seed stage; the hypodermal archesporium cuts off two tapetal cells; the linear tetrad is probably formed; endosperm formation is abundant; the embryogeny is probably of the Capsella-type ; during the first growth of the cotyledons about one-third of the embryo and the greater part of the endosperm are thrust out of the sac and lie in the ovary cavity.—J. M. C.

**Endemic species and mutation.**—WILLIS,[24] in following up the suggestions of his work upon the flora of Mt. Ritigala,[25] has constructed an argument against the origin of species by natural selection and in favor of origin by mutation. He maintains that the evidence of the endemic species on Mt. Ritigala, of Ceylon in general, of Mauritius, and of New Zealand completely proves his position.—J. M. C.

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[21] SCOTT, D. H., The following plants of the Mesozoic age, in the light of recent discoveries. Jour. Roy. Micr. Soc. **1907**: 127–141. *pls. 6–9.*

[22] ROBERTSON, AGNES, The Taxoideae: A phylogenetic study. New Phytol. **6**:92–102. *pl. 1.* 1907.

[23] COOK, MELVILLE THURSTON, The embryology of *Rhizophora Mangle.* Bull. Torr. Bot. Club **34**:271–277. *pls. 22, 23.* 1907.

[24] WILLIS, J. C., Some evidence against the theory of the origin of species by natural selection of infinitesimal variations, and in favor of origin by mutation. Annals Roy. Bot. Gard. Peradeniya **4**:1–15. 1907.

[25] BOT. GAZETTE **43**:353. 1907.

# NEWS

HOMER D. HOUSE has resigned the associate professorship of botany and bacteriology in Clemson College, S. C.

THOMAS D. EASON, a graduate of the Clemson Agricultural College, has accepted a position as assistant in botany at the North Carolina Agricultural College.

Dr. KARL GIESENHAGEN, assistant professor of botany in the University of Munich, has been appointed professor of botany in the Veterinary College at Munich.

THE ADDRESS of Professor FRANCIS E. LLOYD, director of the Department of Investigation, Continental-Mexican Division, Inter-continental Rubber Company, has been changed from Torreon, to Hacienda de Cedros, Mazapil, Zac., Mexico.

CANTERBURY COLLEGE, New Zealand, at the instigation of Professor L. COCKAYNE, has decided to establish a subalpine experimental station in the mountains, where the mountain flora of New Zealand can be studied under natural conditions. The site selected includes a virgin forest, about 300 acres in extent, of *Nothofagus cliffortioides*.

THE MACMILLAN COMPANY announce for publication in the autumn a practical textbook of plant pathology, by D. T. MACDOUGAL, F. S. EARLE, and H. M. RICHARDS; Notes of a botanist on the Amazon and Andes, by RICHARD SPRUCE, edited by ALFRED RUSSELL WALLACE; The physiology of plant production, by B. M. DUGGAR; Bacteria in relation to agriculture, by J. G. LIPMAN; and a new edition of MUIR and RITCHIE'S Manual of bacteriology.

THE GOVERNMENT of New Zealand has planned a magnetic, botanical, zoological, and geological survey of the Auckland and Campbell Islands, the expedition sailing early in November. The botanists will be as follows: L. COCKAYNE, ecologist; T. F. CHEESEMAN, floristic botanist; R. M. LANIG, algologist, and A. H. COCKAYNE, plant pathologist. The survey was first proposed and the details arranged by the Philosophical Institute of Canterbury, a branch of the New Zealand Institute.

THE NEXT MEETING of the American Association for the Advancement of Science will be held during Convocation Week, at the University of Chicago. The sessions of Section G will be held in the Hull Botanical Laboratory. Inasmuch as the Secretary of the Section is at too great a distance for quick mail service, it is particularly requested that titles of papers to be read be sent directly to Dr. HENRY C. COWLES, University of Chicago, Chicago, so that the Secretary, upon his arrival, may have all business promptly in hand.—F. E. LLOYD, *Secretary Section G.*